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The Journal of Infectious Diseases

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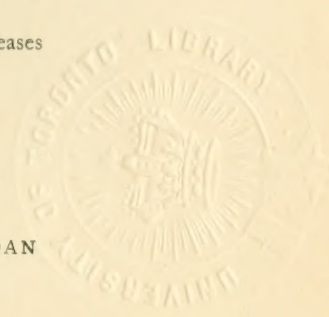
LUDVIG HEKTOEN AND EDWIN O. JORDAN

IN CONJUNCTION WITH

FRANK BILLINGS

F. G. NOVY

W. T. SEDGWICK



Volume 10

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ERRATA.

VOL. 9.

Erratum, "P. 118, line 16," *should read* "P. 116, line 17."

Page 339, line 27, *insert* "(See Table 12)" after "manner."

Page 353, line 30, "Chaulmogra oil or Aesenic" *should read* "Chaulmoogra oil or Arsenic."

Page 463, line 24, "12" *should read* "15."

Page 465, line 3, "visitors" *should read* "visits."

Page 467, Table, Column "Total Cases":

"219" *should read* "221"

"202" " " "200"

"111" " " "112"

"240" " " "250"

"108" " " "109"

"154" " " "152"

"262" " " "261"

Page 468, Table (at top) "Total Primary":

"197" *should read* "199"

"243" " " "241"

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Page 57, footnote 1, "*Münch. Med. Wchnschr.*, 1908, 55, p. 105," *should read* "*Münch. Med. Wchnschr.*, 1907, 47, p. 105."

Page 145, lines 13 and 28, "granulomats" *should read* "granulomas."

The Journal of Infectious Diseases

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January 1912

NO. 1

PYEMIA DUE TO AN ANAEROBIC POLYMORPHIC BACILLUS, PROBABLY BACILLUS FUSIFORMIS.*

E. C. ROSENOW AND RUTH TUNNICLIFF.

(*From the Memorial Institute for Infectious Diseases.*)

The *Bacillus fusiformis*, the anaerobic organism found so frequently in pathological lesions, nearly always occurs in conjunction with aerobic bacteria and some authors even question its pathogenicity. The following case is reported because, so far as we can determine, it is the first fatal general infection caused by this bacillus alone.

The essential facts of the clinical history are as follows:

The patient, a middle-aged man, died from pyemia following an attack of appendicitis. Early operation was refused. An operation five weeks later by Dr. Bevan, to whom we are indebted for the opportunity to make this study, revealed a small pericecal abscess containing a sloughed appendix. Drainage at this time failed to check the septic temperature which the patient had had for some time previously and which continued until death six weeks later. Repeated blood examinations showed a leukocytosis of from 14,000 to 22,000. Three blood cultures were negative. Of these the third was kept under anaerobic conditions. A number of needle punctures of the liver failed to show liver abscess. One month before death metastatic abscesses began to appear over the thigh and tibia and two weeks before death the patient coughed up a large amount of a foul smelling pus.

Anatomic diagnosis (Dr. Rosenow).—Retroperitoneal and retrocecal abscess; thrombophlebitis of contributory retroperitoneal veins; multiple pulmonary infarction with abscess formation; multiple metastatic suppurative periostitis and osteomyelitis; acute pleuritis; infarct of spleen; adhesive localized pleuritis and peritonitis;

* Received for publication November 3, 1911.

fatty infiltration of liver with passive congestion. The examination was made three hours after death. The following statements taken from the postmortem record cover the important points: The peritoneal cavity is free from fluid. The lateral and posterior portion of the cecum is bound firmly to the abdominal wall by fibrous adhesions. The stump of the appendix has entirely healed. On tearing the parietal peritoneum and adhesions along the posterior and outer aspect of the cecum there is opened an abscess from which exudes approximately 200 c.c. of a whitish-yellow, thick, intensely fetid pus. The wall of the abscess is irregular and ragged, easily torn, and contains a number of pockets filled with a similar pus.

On section of the tissues behind and to the left of the abscess a number of veins are cut across which are filled with adherent clots. No evidence of portal thrombosis is found.

The spleen is enlarged, soft, and contains a light-colored, wedge-shaped area ($2 \times 2 \times 1$ cm.) surrounded by a hyperemic zone.

The liver shows a moderate infiltration of fat and passive congestion.

The left pleural cavity is free from fluid. Fibrous adhesions are bound over two medium-sized, wedge-shaped areas on the left upper lobe and over the greater part of the lower lobe. The latter is the seat of a large abscess, containing approximately 500 c.c. of a blood-stained, chocolate-colored, intensely fetid pus. The inner and anterior walls are ragged, irregular, and surrounded by compressed lung, while the lower wall is largely made up of diaphragm. Two wedge-shaped areas of the left upper lobe show softening in the center and are filled with foul smelling pus. An abscess corresponding to the one in the left lower lobe is found just above the diaphragm in the right lower lobe and contains fully 300 c.c. of a similar pus. Four wedge-shaped areas like those found in the left upper lobe are found in the right lung. Cross-section at the apex of some of these areas shows one or more veins filled with adherent clots. No direct communication between the abscesses of lungs and the retrocecal abscess was found.

The stomach, intestines, mesenteric lymph-glands, kidneys, adrenals, pericardium, endocardium, and myocardium show no noteworthy changes.

Microscopic examinations of sections of the wall of the large abscess shows it to consist of necrotic tissue surrounded by granulation tissue and compressed lung, infiltrated with leukocytes. The wedge-shaped areas consist of a central necrotic material surrounded by a hyperemic zone with infiltration of leukocytes.

The liver shows a marked fatty infiltration, a definite increase in interstitial tissue, and passive congestion. No noteworthy change was found in the rest of the organs.

Bacteriology.—Microscopic examination of stained smears of the pus obtained from the abscess of the tibia before and after death, from the large abscess in the left lung, and the retrocecal abscess after death, and from infarcts shows slender, spindle-shaped bacilli, with pointed ends, varying greatly in length (3 to 40μ). They often occur in large masses of long, wavy filaments, twisted together in ropelike fashion and as short spirals (Plate 1, Fig. 1). The twisted threads at times extend across a number of fields of

the oil immersion lens. The ends are usually tapering and curled upon themselves. The threads are undoubtedly made up of many bacilli because at times distinct evidence of a break in angle occurs at the junction of each bacillus. Many small coccus forms are found singly, in pairs, and often in clumps of 50 or more. The masses of cocci are often found near or within the entangled masses of bacilli, while single cocci and diplococcus forms are not infrequently observed near or within a widened portion of the long filaments or single bacilli. There seem to be breaks in one side of these enlarged ends and here the small coccus forms seem to escape.

The pus from the right lung shows the presence of a large number of the same bacilli and coccus forms and in addition a large gram negative bacillus entirely different from those described and which we regard as a contamination, since rupture of the abscess had taken place during life.

No definite motility can be made out although vibratory motion of the ends of the long filaments is seen. The small coccus forms and bacilli are largely decolorized when treated by Gram's method. The bacilli stain with difficulty and the more penetrating stains such as carbol-gentian-violet or carbol-fuchsin are necessary to bring them out clearly.

The fusiform bacillus was isolated in pure culture by growing it anaerobically (Wright's pyrogallic acid method) on slants of blood agar at 37° C. The organism grows on human, sheep, and goat blood agar. Of these the latter is the most favorable medium. The bacillus grows poorly in 0.2 per cent dextrose broth and serum broth. No growth has been obtained on plain agar or Loeffler's blood serum. Growth generally occurs after 48 hours' incubation at 37° C.

The colonies are grayish-white in color, rather shiny, and usually have regular edges. When old, the colonies frequently become indented in the center and flattened at the edges. In early cultures, especially, the colonies are quite adherent to the medium. The colonies vary from the size of a pin-point to 2 mm. in diameter. Single colonies always contain fusiform bacilli and very small gram negative coccus forms which are often in pairs and clumps, just as in the smears from the pus. In the fluid of condensation the

organisms often grow in yellowish-white balls. The blood agar is often colored green, especially near the fluid of condensation.

An offensive odor is given off in successful cultures.

No progressive motion could be observed, but a distinct vibratory motion is seen, especially at one end.

The bacilli die quickly. It is very difficult to get a growth from a single colony, only about one out of 10 such cultures being successful. To assure growth a large amount of pus or culture must be inoculated. It was never possible to separate the coccus forms from the fusiform bacilli or to get growth from colonies containing mostly coccus forms and few bacilli. The coccus forms also grow only in anaerobic cultures which differentiate them from *melitensis* which they resemble. The bacillus is very polymorphous. The forms seen in pure cultures closely resemble those seen in the pus. In the cultures the proportion of bacilli and short spiral forms is generally greater than in the smears made from the pus. In pure culture the organism appears as straight, or slightly bent, pointed bacilli of varying lengths, some being very short, long, wavy filaments, ropes, long and short spiral forms, and coccus forms. The filaments sometimes form a circle, assume U-shapes and occasionally have a curved end. The coccus forms are usually found near the twisted threads and at times are seen inside or escaping from swollen ends, just as seen in the pus. Occasionally they appear in short chains. No spores can be demonstrated in these cultures. Involution forms in a great variety of shapes are sometimes seen in the old cultures. Distinctly spiral forms are seen only in the fluid of condensation in the early cultures, but appeared on the surface of the solid media after six weeks' cultivation. The filaments and spiral forms are often seen to be made up of strings of bacilli as in the smears from the pus (Plate 1, Fig. 1). The spiral forms are best seen in the hanging drop, India ink preparations, and in smears fixed by heat or with methyl alcohol and then stained with carbol-gentian-violet or polychrome methylene blue (24-48 hours). A 10 per cent saturated solution of gentian-violet in 5 per cent phenol was found the most satisfactory stain. It is not necessary to heat the stain. The organisms are not positively stained by Gram's method.

This bacillus corresponds closely, culturally and morphologically, with those isolated in pure culture by Tunnicliff¹ from the normal mouth, noma, membranous angina, and gingivitis. The only differences are the coccus forms, which resemble somewhat the spores described by Tunnicliff, and the cup-shaped colonies in old cultures. Morphologically Tunnicliff's organism is extremely polymorphous, appearing as short bacilli, filaments, long and short spiral forms.

These strains of fusiform bacillus appear to be similar to that of Kasper and Kern.² The difference in culture media probably causes greater production of spiral forms in the cultures here described. The predominance of long ropes and filaments in the pus from the internal organs may be due to a greater degree of anaerobiosis than is found in the mouth, in noma, membranous angina, and gingivitis, where short spirilla are observed. The cases of pyemia in which long filaments and ropes are found are much more chronic than cases of noma and Vincent's angina, and the long period of growth in the fluids of the same individual may account for the large number of filaments and long spirals instead of the short forms. This is in accord with the observations of Tunnicliff that the filaments and spirals are a later stage in the development of the fusiform bacillus.

The chronicity of infection in these cases may explain also the greater adherence of the colonies to the surface of blood agar just as Rosenow³ has shown in cases of chronic pneumococcus endocarditis in which the property of adherence and chain formation is probably the result of long growth in serum rich in agglutinins.

Animal experiments.—The virulence of the microorganism was tested on white rats, rabbits, and guinea-pigs. Two white rats which received four daily intraperitoneal injections of 1 c.c. of pus remained permanently well. Two guinea-pigs which were injected intraperitoneally with 0.5 c.c. of the fresh pus from the left lung died at the end of 48 hours. No gross lesions or bacteria could be found after death. Two guinea-pigs injected repeatedly with the

¹ *Jour. Infect. Dis.*, 1906, 3, p. 148; *ibid.*, 1911, 8, p. 316.

² *Centralbl. f. Bakt.*, I, Orig., 1910, 55, p. 97.

³ *Jour. Infect. Dis.*, 1910, 7, pp. 411, 429.

pus after it was kept on ice for 48 hours and longer remained well. It was thought that repeated injections of the pure culture might be of interest and accordingly two rabbits were injected intraperitoneally with the growth from two to six blood agar slants on successive days. They died in four and 13 days respectively. In one peritonitis was found and the bacillus easily made out in smears from the flakes of fibrin, but the cultures unfortunately remained sterile. In the other no gross lesions were found. A third rabbit having previously received four daily intravenous injections of 1 c.c. of pus was not made noticeably ill by three large doses of the pure culture. It seems as if the injections of the attenuated pus conferred a definite immunity. One rabbit and one guinea-pig, injected subcutaneously with the contaminated pus from the right lung abscess, died in three days. Microscopic and cultural examination of the pus, which had infiltrated the subcutaneous tissue over a large area, proved the presence of a large number of fusiform bacilli together with the gram negative saprophytic aerobic bacillus isolated previously and which proved nonvirulent for a guinea-pig. The small coccus forms described above were found both in the pus and in cultures. No metastatic abscesses could be found anywhere.

Ghon and Mucha,¹ and Kasper and Kern report similar cases where the exact etiological rôle of a similar bacillus, which they believe to be the bacillus fusiformis, is not quite so clear because it was not found in pure culture. Our results appear to show that the bacillus fusiformis by itself may be pathogenic.

EXPLANATION OF PLATE 1.

FIG. 1.—Pus from retrocecal abscess, showing long threads, ropelike twists, short spiral, and coccus forms. Carbol-gentian-violet. $\times 1,000$.

FIG. 2.—Pus from abscess in left lung. Carbol-gentian-violet. $\times 750$.

FIG. 3.—Culture of fusiform bacilli from pus in right lung after 48 hours' growth on goat blood agar. Carbol-gentian-violet. $\times 750$. (The large thick bacillus is a contaminating bacillus from which the cultures of fusiform bacilli were freed later.)

FIG. 4.—Smear showing straight and curved bacilli and spirals from 48 hours' growth on goat blood agar. Carbol-gentian-violet. $\times 1,000$.

¹ *Centralbl. f. Bakt.*, I, Orig., 1909, 49, p. 493.

PLATE 1.

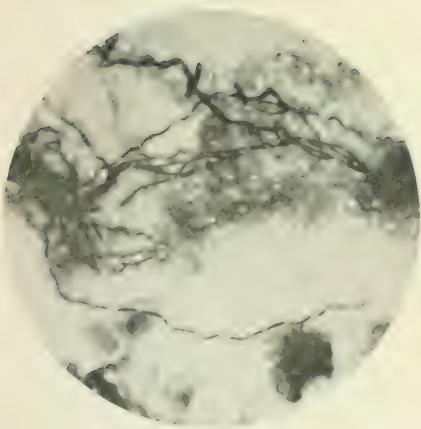


FIG. 1.

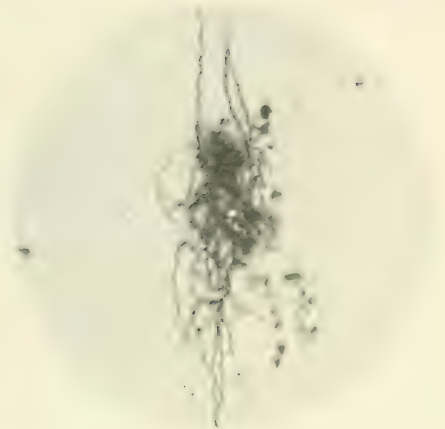


FIG. 2.

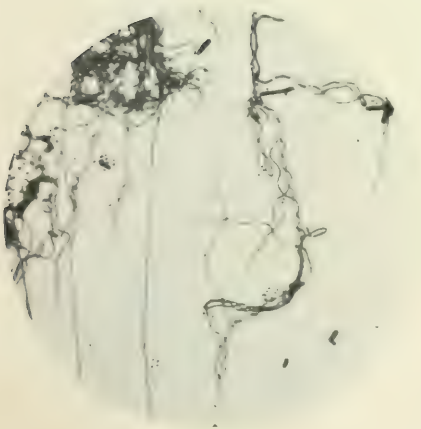


FIG. 3.

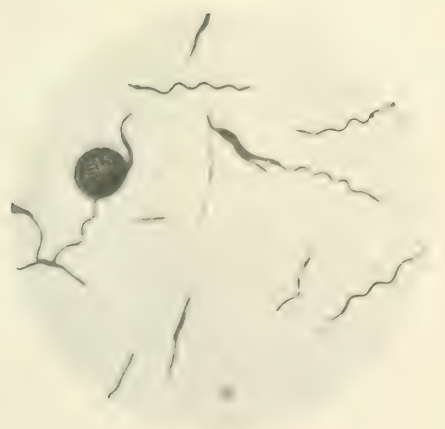


FIG. 4.

THE CELL CONTENT OF MILK.*

H. E. ROSS.

(From the New York State College of Agriculture at Cornell University, Ithaca, New York)

During the past few years the cell content of milk has received considerable attention. Those who first discovered the fact that milk contained cells supposed these cells were leukocytes. Whenever there is inflammation or suppuration there are always large numbers of pus cells, and according to Bergey a pus cell is simply a dead leukocyte. For the reason that leukocytes are found under inflammatory conditions it was supposed that the presence of these cells in milk indicated an inflammatory condition of the udder. While most investigators agree that some of the cells found in milk are leukocytes it is by no means certain that some of them are not epithelial cells. It was supposed that the number of cells found in milk was of prime importance in determining the presence or absence of inflammation.

There are two chief methods which are usually employed to enumerate the cells in milk. One of these is called the smeared-sediment method and the other is called the Doane-Buckley, or volumetric, method.

The smeared-sediment method was devised by Stokes of the Baltimore Board of Health, and was later modified by Stewart of the Philadelphia Board of Health, and Slack of the Boston Board of Health.

Briefly described, the smeared-sediment method consists in centrifugalizing one or two cubic centimeters of milk. The containers of the milk are small glass tubes closed at each end with a rubber stopper and the centrifuge is run at a high rate of speed. The sediment obtained is spread over a definite area on a cover glass, stained, and the number of cells counted.

The volumetric method was adapted by Charles F. Doane and S. S. Buckley at the time, respectively, dairyman and veterinarian at Maryland Experiment Station. The comparative results

* Received for publication August 28, 1911.

obtained by the smeared-sediment and volumetric methods have been studied by Doane, Russell and Hoffman, of Wisconsin, and Ward, of California. All are unanimous in pronouncing the volumetric method the most accurate one.

In the study of the cell content of milk we used only the volumetric method somewhat modified. The work done may be particularly divided into two parts: first, a study of the volumetric method of enumerating cells in milk; and second, a count of the cell content of milk in different cows and under different conditions for various lengths of time.

VOLUMETRIC METHOD OF ENUMERATING CELLS IN MILK.

Since this method was considerably modified we will describe it as it was used. Ten c.c. of milk were centrifugalized for 10 minutes in a graduated glass tube after heating to a temperature of 100° F. to 120° F. The heating was accomplished by immersing the tubes in warm water. A direct flame is liable to crack the tubes. Care should be taken not to heat the milk highly enough to precipitate the albumen, which would obscure the lines in the counting chamber. The centrifuge had a diameter of 13 in. when the cups were extended and ran at a speed of 2,000 revolutions per minute. With a machine of this diameter it was found impractical to run at a speed of less than 1,800 revolutions per minute. This speed was necessary not only to throw down the cells but to remove all of the fat. After centrifugalizing for 10 minutes the fat was removed by means of a cotton swab. The tubes were then centrifugalized for one minute more and the remaining fat was removed. In whirling the tubes a second time, care should be taken to have the liquid at the same level in each tube, as otherwise the machine will be unbalanced. All of the liquid possible was then withdrawn. Care should be taken not to disturb the sediment in the bottom of the tubes and for this reason it is impossible to withdraw all of the liquid. In most cases it was withdrawn to the 0.5 c.c. mark on the tube. In withdrawing this liquid we used a bent pipette the intake of which was on the side of the tube instead of at the end and this opening was about a quarter of an inch from the end of the tube. The bent pipette was convenient because it allowed the

operator to hold the tube in such a position that it could readily be seen when all of the liquid necessary had been removed.

After the supernatant liquid was withdrawn a stain was added. The stain was made by mixing 15 drops of a saturated gentian-violet solution with 100 c.c. of a 0.6 per cent solution of sodium chloride. This solution tinted the cells a faint purple and yet did not stain everything such a deep color as to make some of the objects in the counting chamber unrecognizable. Some stains did have this objectionable feature. Russell and Hoffman used no stain but we found this proved rather hard on the eyes, and in some cases it was difficult to identify objects under the microscope unless the stain was used. A good example of such an object would be a piece of casein with a fat globule entangled in it. The sediment is usually packed so hard in the bottom of the tube that a platinum needle or some similar instrument is necessary to break up the sediment. Enough of the stain was added to make the volume of liquid up to one c.c., which made it an easy basis of calculation. After the stain was thoroughly mixed with the sediment a drop of the mixture was transferred to the counting chamber and the cells enumerated. The drop of liquid in the chamber was rejected and a new one taken under the following conditions:

1. If the cover glass did not fit snugly against the slide. This would be due to foreign material underneath the cover glass.
2. If the drop did not cover the entire ruled area.
3. If the liquid overflowed the counting chamber and ran between the cover glass and the slide.
4. If on examination the cells were not fairly evenly distributed over the entire field.
5. If any air bubbles were under the cover glass.
6. If any foreign material was found in the chamber which would cover up the cells. This might include precipitated casein, particles of skin from the udder of the cow, and fat globules.

In using the counting chamber it is best to have some definite method of enumerating cells and always use that method. This will save much useless confusion. For instance, we always began at the upper left-hand corner of the counting chamber and worked

downward, counting the four blocks of $\frac{1}{16}$ sq. mm. each. We then began at the bottom and counted upward on the squares at the extreme right hand of the ruled area. The next four squares were counted working downward, and then beginning again at the bottom, the four remaining squares were counted. There is no particular reason for this method except that it is a fixed one, and by setting down the number of cells in the order in which they were counted one could tell at a glance how many cells were in any particular square without enumerating them again.

METHOD OF COMPUTING RESULTS.

The results obtained were expressed in terms of cubic centimeters. We will suppose, for example, that there were in the entire field 55 cells. Since the chamber is 0.1 mm. deep the 55 was multiplied by 10, making 550 cells per c.mm. There are, however, 1,000 c.mm. in a c.c., so that to reduce our results to c.c. the 550 was multiplied by 1,000, making 550,000 cells. However, we started with 10 c.c. of milk and counted and concentrated in one c.c. of milk all of the cells there were in 10 c.c. The results are, therefore, 10 times too large, and dividing 550,000 by 10 we get a result of 55,000 cells per c.c.

The practice of counting part of a field and then estimating the number of cells in the entire chamber is sometimes followed. Since the counting chamber contains 16 squares, either four or eight squares are usually counted and the results then multiplied by four or two respectively. In order to see how accurate such an estimate would be, 100 samples were counted in which a comparison of counting four squares, eight squares, and the entire field was made. When four squares were counted and an estimate made there was an average variation of 8.89 per cent. When eight squares were counted and an estimate made there was an average variation of 6.57 per cent. A count of the entire field was of course taken as a basis for computing the percentage of variation.

In order to test the accuracy of the volumetric method, two counts each were made of 56 samples of milk. There was found to be an average variation of 12.76 per cent between the two counts. This is considerably higher than the results obtained by

Russell and Hoffman, who found a variation of only 5.6 per cent. There were, however, included in these counts three or four abnormally high variations. One variation was over 55 per cent. While these variations were abnormal and greatly increased the average variation, it was thought best to include them because they show the variations which it is possible to obtain by using this method.

In separating milk with a centrifugal separator the milk is heated before separation in order to make the removal of the fat more thorough. The presence of fat globules in the counting chamber is a disturbing factor and it was thought only reasonable that warming the milk before centrifugalizing would help to remove the fat more thoroughly. A trial proved this to be the case, but also showed that milk which was warmed before centrifugalizing gave a much higher cell count than milk which was not warmed. This was probably due to the fact that warming milk expanded it and therefore made it easier to throw the cells to the bottom of the tube. Two counts each of 50 samples were made, one from a heated sample and one from an unheated sample. The unheated samples were centrifugalized at a temperature of from 60° to 70° F. and the temperature of the heated samples was 100° to 120° F. The samples which were heated before centrifugalized gave an increase in cell content of 13.6 per cent. Care should be taken not to heat the samples hot enough to precipitate the albumen, as this would obscure the ruled area in the counting chamber.

VARIATIONS IN MILK FROM DIFFERENT COWS.

In all of the samples examined, no milk was found free from cells. In an examination of the milk from 50 different cows the cell content varied from 4,000 to 3,576,000. To all appearances both cows from which these samples were taken were perfectly normal and healthy, and there was no apparent reason for the difference in count.

COMPARATIVE COUNTS OF FORE MILK, MIDDLE MILK, AND LAST MILK.

In this experiment, the first 10 or 15 streams from each teat were regarded as the fore milk. The middle milk was obtained when the cow was approximately half milked, and the last milk

consisted of what is ordinarily termed the "strippings." Fifteen different samples were compared and in every case the last milk contained the greatest number of cells per c.c. In 11 samples of the 15 counted, the first milk gave the smallest count, the middle milk next highest, and the last milk highest. The percentage increase in cell content per c.c. of the last milk over the fore milk was 53.85, and that of the last milk over the middle milk was 52.14.

EFFECT OF MANIPULATION OF THE UDDER.

It is not definitely known whether the cells found in milk are true leukocytes or whether they are epithelial cells. In either case it seemed reasonable to suppose that manipulation of the udder would tend to increase them. The manipulation would break down cells and if vigorous enough to produce inflammation would cause an increase in leukocytes.

The method of conducting this experiment was as follows: A count of the milk was taken at night and on the following morning. On the following evening, 24 hours after the first count, the udder was vigorously manipulated for five minutes. The cow was then milked and a count of the milk made.

Generally speaking, the results of this experiment were unsatisfactory. In the majority of cases there was an increase in cell content after the udder was manipulated, but this increase was sometimes so small that it was uncertain whether the increase was due to manipulation or some other causes. Nine experiments were made and the average percentage of increase was 11.98 for the first count and 35.29 for the second count. In a small percentage of the nine experiments there was a decrease in cell content per c.c. after the udder was manipulated.

VARIATIONS IN A LONG PERIOD.

In studying the cell content of milk for a long period of time three cows of different breeds were selected. Golden Daisy was a Jersey and freshened October 6, 1908. Sigma was a Holstein and freshened October 8, 1908. Lady Benton was a Shorthorn and freshened October 10, 1908. The milk from the first two was examined for a period of a little over seven months. Lady Benton

was sold for beef before the experiment was concluded and her milk was examined for a period of about five months only.

Several items of interest were noted in the examination of the milk for this long period of time. In the first place, the cell content of the milk per c.c. varies both upward and downward, but the variation in individual cows was within certain limits. In the case of Golden Daisy, the cell content of the milk varied normally from 4,000 to 99,000, but was usually between 40,000 and 80,000. The milk from Sigma was not so uniform in cell count as was that from Golden Daisy, varying from 21,000 to 272,000; yet the count of the milk from Sigma was normally higher than that from Golden Daisy, and the milk from the former varied in cell content normally from about 40,000 to 150,000.

The milk from Lady Benton usually gave a very high count. The number of cells varied from 107,000 to 1,063,000. The count rarely fell much below 300,000 and was usually much higher.

The counts from these three cows present a wide variation for which we cannot account. It seems certain that this variation is not due to breed. Another Shorthorn cow gave a count of 18,000 cells per c.c. It was our general experience that the individual count varied without regard to breed.

During the two weeks while this experiment was in progress, Golden Daisy was ill of indigestion. During this time the amount of milk she gave was very small and the cell content per c.c. was abnormally high. One week the cow gave 25.5 pounds of milk and the second week 17.6 pounds of milk. The cell count for these respective weeks was 988,000 and 314,500 cells per c.c. The high cell content may perhaps be accounted for on the grounds of inflammation of the udder.

RELATION BETWEEN FAT CONTENT AND CELL CONTENT OF MILK.

It was suggested that cows giving milk with a high percentage of fat might yield milk of a higher cell content than those giving milk with a low percentage of fat. It is fairly well known that in the process of milk secretion the cells of the acini are very active and are rapidly disintegrated; it therefore seemed reasonable that the more fat in milk, the greater number of cells there would be.

On investigation this theory did not hold good. The average percentage of fat found in the evening milk of Golden Daisy was 6.05 and the average cell content of the milk per c.c. was 82,214.

The average percentage of fat found in the evening milk of Sigma was 3.7 and the average cell content of the milk per c.c. was 118,681. The milk from Sigma averaged 2.35 per cent less fat than did the milk from Golden Daisy; yet the milk from Sigma contained 36.467 more cells per c.c. than the milk from Golden Daisy. It was also found that the milk from Lady Benton contained less fat than the milk from Golden Daisy, yet the milk of the former had a higher cell content than that of the latter.

On the other hand the results did not show that a low percentage of fat necessarily indicated a high cell content. Sigma gave milk lower in fat than did Lady Benton and yet her milk had a lower cell content.

Results obtained from the examination of milk from other cows were the same as in the case of the three cows mentioned above.

In order to test this point still further, those tests which were above the average percentage of fat were themselves averaged. Then the cell counts corresponding to the percentages of fat above the average were averaged. The results obtained indicated that the cell content had no relation to a high percentage of fat.

It is possible, however, that the percentage of fat present in the milk and the number of separate globules which go to make up that percentage have no definite relation. It is well known that the fat globules in milk from different breeds of cows vary in size. It is reasonable to suppose that even though the percentage of fat in milk was low, the number of fat globules might be large, and in consequence the cell content might be high.

RELATION OF NUMBER OF CELLS TO QUANTITY OF MILK PRODUCED.

It has been pointed out already that under normal conditions the cell content of milk per c.c. from an individual cow is fairly constant. By comparing the cell count of the milk with the number of pounds given, we are led to conclude that the total number of cells decreases as the quantity of milk decreases.

During the week of October 18, 1908, Golden Daisy gave 195.6

pounds of milk; during the week of April 19, 1909, she gave 83.7 pounds of milk. During the week of October 13, 1908, Sigma gave 263.8 pounds of milk, and during the week of April 19, 1909, 203.6 pounds of milk. During the week of October 14, 1908, Lady Benton gave 156.9 pounds of milk, and during the week of February 16, 1909, she gave only 21.6 pounds of milk. In each case 10 c.c. of the milk were examined, and yet we found that the number of cells per c.c. obtained at the last count was neither abnormally high nor abnormally low. Unless the number of cells decreased as the milk decreased, we would get an abnormally high number of cells per c.c. in the last count.

COMPARISON OF COLOSTRUM AND NORMAL MILK.

In the following table is given a comparison of the cell content of colostrum with the cell content of milk from the same cow later in the lactation period. The table needs no special explanation. It will be seen that the cell content of colostrum was always higher than was the normal milk later in the lactation period. This is what would naturally be expected because of the sudden activity of the udder after a period of inactivity. Apparently most of the cells present in colostrum are broken-down epithelial cells.

TABLE SHOWING A COMPARISON OF THE CELL CONTENT OF COLOSTRUM WITH THAT OF NORMAL MILK.

Sample Number	Colostrum Cells per c.c.	Number of Milking	Normal Milk Cells per c.c.
1.....	713,500	8th	18,000
2.....	3,935,000	7th	13,000
3.....	332,000	3d	20,000
4.....	500,000	6th	68,000
5.....	553,000	5th	49,000
6.....	332,000	21st	20,000

EFFECT OF AN INJURED QUARTER OF THE UDDER.

In the midst of our investigations of this subject, one of the cows in the herd suddenly began to give milk that was decidedly abnormal. An examination showed that one quarter of the udder was affected. Thereafter the milk from this quarter was drawn in a

vessel separate from the milk of the other three quarters. A cell count was made of the two samples with the following results:

Infected quarter, 62,400,000 cells per c.c.

Three sound quarters, 407,000 cells per c.c.

These two counts are interesting because they show that one quarter may be seriously affected without similarly affecting the other three quarters.

LEUKOCYTE STANDARDS.

There has been much recent discussion regarding the establishment of a standard for the cell content of milk, called a leukocyte standard. It has been pointed out that in many cases it is almost impossible to distinguish between leukocytes and the other cells. Some investigators have suggested that the maximum number of cells allowed should be 100,000 per c.c. From the results obtained in this investigation, it would seem unwise to set any standards for the cell content of milk until we have more definite information as to causes and effects. If the standard of 100,000 cells per c.c. were enforced, the milk from two of the three cows here considered would have to be rejected; yet these animals were, so far as we were able to learn, perfectly normal and healthy. It has been shown that the total number of cells seems to decrease as the quantity of the milk decreases. This would indicate that the formation of cells, or leukocytes, is a normal function of milk production.

BACILLUS MURIS AS THE ETIOLOGICAL AGENT OF PNEUMONITIS IN WHITE RATS AND ITS PATHOGENICITY FOR LABORATORY ANIMALS.*

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B. muris was first described by Klein¹ in 1900 as the chief organism found in two cases of pneumonia of white rats. The original report is as follows:

Klein described a new pathogenic microbe belonging to the group of diphtheroid bacilli. It was found in white rats, causing in these animals extensive consolidation of the lungs. In the hepatized parts, the alveoli and the infundibula and bronchi were filled with and distended by fibrinous exudation, red and white corpuscles, and continuous masses of the above microbe. Microscopic specimens and lantern slides of the microbe, as it occurred in the lungs and under cultivation, were shown. The morphological, including Neisser's test, and cultural characters, including acid production, of the microbe appeared identical with those of *B. diphtheriae*. Small and large doses of culture caused, on inoculation into rodents, a firm tumor, which gradually enlarged to considerable size, and in the course of a fortnight led to the formation of an abscess. This eventually opened spontaneously and led to complete recovery. No fatal issue in rats or guinea-pigs had been produced by even large doses of the culture. Diphtheria antitoxin, 400 or even 600 units per half-grown guinea-pig, had no power to neutralize the pathogenic action of a small dose of the culture.

The present work was begun in the fall of 1909 at the request of Dr. C. M. Jackson, who had been greatly hampered in certain investigations on white rats because of the death of many of the animals. A routine examination of the dead rats resulted in the constant finding of pneumonitis with consolidation and abscess formation. Some of the lungs did not show distinct abscess formation but only areas of consolidation, resembling lobar pneumonia, with the bronchi filled with a ropy, tenacious mucus and fibrin. The majority, however, were accompanied by abscess formation. These abscesses varied in size from a pin's head to 2 cm. in diameter. They were scattered throughout the lungs and many times occupied an entire lobe. Most of these abscesses were filled with a cheesy pus, although some were filled with a ropy

* Received for publication October 30, 1911.

¹ *Lancet*, 1903, I, p. 238; *Centralbl. f. Bakt.*, I, Orig., 1903, 33, p. 488.

mucus like that which was so frequently found in the bronchi. Upon microscopical examination of consolidated areas from rats, dead early in the course of the disease, a bronchopneumonia was found. The bronchi were distended with fibrin, leukocytes, mostly of the polymorphonuclear variety, and desquamated lining cells. The alveoli were filled with the same sort of exudate. Many of these lining cells contained hemosiderin. The capillaries were distended. Sections from the larger consolidated areas of the older cases showed, for the most part, a caseous degenerated material with chromatin detritus scattered through it.

The number of bacteria in smears from all of these lesions varied considerably. In some cases there were few or none, while in others there were numerous bacteria, for the most part in the shape of short granular rods.

Cultures made from the diseased lungs gave an almost constant finding. Blood serum was used for immediate cultures and in nearly all instances the growth, if any, was a pure culture of *B. muris*. The morphology of this organism varied. Fresh cultures showed a short bacillus varying from 1.5μ to 2.5μ in length, most of them having an unstained band. Polar granules, or in some cases three to five scattered granules, were present. Others showed two or more unstained bands and, in some cultures, especially in those under cultivation for some time, granular forms predominated. The organism was slightly larger at one end, and the form having one unstained band resembled very closely, at first sight, *Str. pneumoniae*. Some solid forms were nearly always present. In very old cultures branching forms were found. These branches were simple lateral offshoots and various bizarre forms.

STAINING.

B. muris stained well with the ordinary dyes, particularly Loeffler's methylene blue. The granules were well stained by Neisser's method. The organism was gram positive.

CULTURAL CHARACTERISTICS.

Broth.—There was clouding of the media in 24 hours. Upon standing, the bacilli collected at the bottom of the tube. When the tube was shaken they readily diffused through the medium.

ANIMAL EXPERIMENTS.

No. of Animal	Method	Kind of Animal Dosage	Date Inoculated	Date Killed or Dead	Pathological Findings	Cultures
1.....	1	Wild mouse, 0.5 c.c. 24-hour broth culture	6/25/10	6/28/10 Found dead	Lungs congested	Negative
2.....	1	Wild mouse. Same as No. 1	6/25/10	6/28/10	Lungs congested	Negative
3.....	1	Guinea-pig. Same as No. 1	6/25/10	7/21/10 Killed	Negative	Negative
4.....	1	White rat. Same as No. 1	6/25/10	7/1/10 Killed	Negative	Negative
5.....	1	White rat, 0.5 c.c. blood serum culture	7/2/10 7/7/10	7/25/10 Killed	Negative	Negative
6.....	2	White rat, 1 c.c. 24-hour blood serum culture	6/30/10	7/21/10	Abscess formation at site of inoculation discharging a creamy pus	Positive
7.....	2	Common mouse, 0.5 c.c. 24-hour blood serum	6/30/10	7/25/10 Found dead	Lungs consolidated. Two abscesses in the ventricles of the heart. Large abscesses on the diaphragmatic surface of the right lobe of liver, large abscess in the head of the spleen, multiple abscesses in the kidneys	Positive from, lungs, liver, spleen, kidney, and heart
8.....	2	Common mouse. Same as No. 7	7/16/10	7/25/10 Killed	Abscess at the site of inoculation	Positive
9.....	2	White rat, few drops of 48-hour broth culture	7/18/10	7/25/10 Killed	Abscess at the site of inoculation measuring 1 cm. in diameter	Positive
10.....	4	Guinea-pig 1 c.c. 48-hour broth culture	7/18/10	7/25/10	Induration at the site of inoculation and abscess formation	Positive
11.....	3 and 4	White rat, 0.5 c.c. 48-hour broth culture	7/19/10	7/22/10 Found dead	Yellowish-white purulent collections scattered over the peritoneum. Pin head in size. Granular fibrino-purulent exudate causing adhesion of pleurae of both lungs. Abscess in the wall of the thorax at the site of inoculation. Deep red consolidated areas in both lungs. Fibrino-purulent pericarditis	Positive from peritoneum, pleura, kidney, and lungs
12.....	3 and 4	Guinea-pig, 0.5 c.c. 24-hour agar culture	10/1/10 10/12/10	11/1/10 Killed	Negative	Negative
13.....	2	Guinea-pig. Same as No. 12	10/1/10	11/1/10	Abscess following at site of injection which healed spontaneously. Otherwise negative	Negative when killed

ANIMAL EXPERIMENTS—Continued.

No. of Animal	Method	Kind of Animal Dosage	Date Inoculated	Date Killed or Dead	Pathological Findings	Cultures
11.	3 and 4	Domestic rat, 0.5 c.c. 24-hour agar culture	10/1/10	10/7/10 Found dead	Small abscess in the abdominal wall. Large abscess in the right thoracic wall. Granular fibrino-purulent exudate causing adhesion of pleurae of both lungs. Lungs congested and pneumonic. Fibrino-purulent pericarditis	Positive from pleura, peritoneum, and heart's blood
15.....	5	Guinea-pig	10/9/10	12/5/10 Killed	Negative	Negative
16..	4	Domestic rat, few drops of 24-hour agar culture	11/7/10 11/17/10	12/7/10 Killed	Abscess formation few days after first injection discharging creamy pus. Discharging fistula connecting with subcutaneous abscess and abscess in right lung measuring 1 cm. in diameter	Positive from site of inoculation and lung abscess
17..	4	Domestic rat, 1 c.c. agar culture	11/17/10	12/2/10 Found dead	Abscess measuring 4 mm. in upper pole of right kidney	Positive
18.....	3	Domestic rat, 1 c.c. agar culture	11/17/10	11/24/10 Found dead	Abscess in thoracic wall. Granular fibrino-purulent exudate causing adhesion of the pleurae of both lungs. Pneumonia. Fibrino-purulent pericarditis	Positive from site of inoculation, lungs, and heart's blood
19.....	3	White rat, 0.5 c.c. 24-hour agar culture	1/12/11	1/14/11 Found dead	Bilateral granular fibrino-pleuritis. Lungs consolidated. Fibrinous pericarditis	Positive from pleural cavity and heart's blood
20, 21, 22	6	White rats, 1 c.c. filtrate of 10-day lactose broth culture in right pleural cavity	2/3/11	3/10/11	Negative	

Gelatin stab.—A granular streak along the line of inoculation; no liquefaction.

Agar streak.—The organism grew along the line of inoculation as very small white granular colonies with a smooth border and with little tendency to coalesce.

Blood serum.—Granular colonies similar to those on agar, but larger. Where widely separated, the colonies became much larger.

Hiss's serum water.—Acid reaction in dextrose and maltose, but not in saccharose and lactose.

Litmus milk.—No change.

ANIMAL EXPERIMENTS.

For clearness in the table of experimental data, each method used will be described and given a number.

1. Suspensions of the organism were injected into the mouth and nose of the animal in an attempt to produce lesions in the lungs by inspiration.

2. Subcutaneous injections of bacillary suspensions were made for the study of the local reaction and in an attempt to produce a general infection.

3. Intrapleural injections of bacillary suspensions were made in order to study the reaction of the pleura and lung tissue.

4. Intraperitoneal injections of bacillary suspensions were used to study the effect on the abdominal viscera.

5. Moistening the food with bacillary suspensions, to see if the disease was transmitted in this manner.

6. Injections of the filtrate of lactose broth cultures, to see if a soluble toxin was produced.

GENERAL DISCUSSION.

With method 1, all of the animals gave negative results. With method 2, however, positive results were obtained in every case. Most of the animals had the same lesion as described by Klein, but the injection into animal 7 resulted in a septicemia and general infection. The results of intrapleural injections established the fact that *B. muris* was fatal to rats when injected at the proper site. Those dying as a result of intrapleural injections showed the same lesions; namely, a granular fibrino-purulent exudate causing adhesion of the visceral to the parietal pleura, consolidated areas in the lungs, and a granular fibrino-purulent pericarditis. The results obtained in animal 16 resemble more closely the natural disease. That the animal did not die was due to the establish-

ment of a permanent fistulous tract between the lung and the outside world. The extent of lesions in animal 17 is rather insignificant to produce a fatal result, yet the presence of a nephritic abscess from which a pure culture of *B. muris* was obtained must be accepted as the cause of death. None of the animals injected with the lactose broth filtrate gave any evidence that a soluble toxin was produced.

Microscopical examination of the so-called "abscesses" which occurred in the kidneys, spleen, and liver, and the granular collections on the pleura, pericardium, and peritoneum showed them to consist mainly of a growth of *B. muris*. These findings are very similar to the "drüsen" formation of actinomycosis and also to the lesions produced by many bacteria belonging to the group of diphtheroid and pseudo-tuberculosis bacilli.

Many diphtheroid bacilli and pseudo-tuberculosis organisms are pathogenic for the lower animals, particularly rodents. They are all very similar to *B. muris*. The bacillus of pseudo-tuberculosis of mice is described by Kutscher¹ as follows: "Morphologically, it resembles the diphtheria bacillus, staining irregularly, appearing in bizarre forms, grows readily, does not form spores, does not liquefy gelatin, is gram positive, and proved pathogenic only for mice." Reed² in working with this same organism confirmed the findings of Kutscher. Part of her summary is as follows:

1. We may conclude that several different bacteria are concerned in the bacillary pseudo-tuberculosis of animals.

2. Rodents seem especially liable to such forms of tuberculosis.

3. The bacilli described by Kutscher and Welch are identical.

4. The experimental production of the natural disease in mice can be accomplished by injections of pure cultures of the bacilli. The only certain methods are inoculation into the pleural and peritoneal cavities.

5. The pseudo-tubercles of the disease differ from those previously described in being composed essentially of colonies of bacteria and to a small extent, only, of proliferated and emigrated body cells. The propriety of the denomination "pseudo-tuberculosis" is therefore open to question.

6. The bacilli occur in the form of simple and branching rods; the branching is observed in growths in the animal and those in artificial culture. The bacillus closely resembles, both morphologically and in culture, the Klebs-Loeffler bacillus, but can be distinguished from the latter.

¹ *Ztschr. f. Hyg.*, 1894, 18, p. 327.

² *Contributions to the Science of Medicine by the Pupils of William H. Welch*, Baltimore, 1900.

B. muris differs from the above in the following ways: It is stained nicely by Neisser's method, it is pathogenic for the wild mouse, domestic rat, guinea-pig, and the white rat.

Purulent lesions are very common in the lungs of rodents to which, as yet, no specific organism has been assigned. McCoy and Currie¹ describe these lesions in a bulletin issued by the Public Health and Marine Hospital Service. An extract from a letter from Dr. McCoy is as follows: "On several occasions I have inoculated guinea-pigs from purulent lung lesions of rats but always with negative results. I thought it just possible that they might represent the lesions of plague. This, however, was not the case. Purulent lesions of the lungs are very common among rats here. I should say that at least one per cent of those examined in San Francisco are affected in this manner."

B. muris was found only in the lungs of rats that were suffering from pneumonia. In many of these animals abscesses were also present, but it could not be said that *B. muris* caused the abscesses. In animals in which no abscesses existed, but only the pneumonic condition, *B. muris* was isolated in pure culture. Since the beginning of this investigation the fatal epidemic among the white rats has disappeared. Many of the remaining animals, killed for other research work, show abscess formation in their lungs but no pneumonia. In none of these has it been possible to isolate *B. muris*.

CONCLUSIONS.

1. *B. muris* is the etiological agent in a specific kind of pneumonitis in white rats.
2. *B. muris* is pathogenic for the white rat, the domestic gray rat, the common mouse, and the guinea-pig.
3. In the case of the guinea-pig the infection remains local.
4. Instances of a general infection occur, as in animal 7.
5. *B. muris* belongs to the group of diphtheroid or pseudo-tuberculosis bacilli.

In this investigation I have been greatly aided by suggestions from Dr. C. M. Jackson of the University of Missouri and Dr. Edwin O. Jordan, of the University of Chicago, and I take this opportunity of thanking them.

¹ *Pub. Health Bull. No. 30*, U.S. Public Health and Marine Hospital Service, Washington, 1910.

REPORT OF SOME EXPERIMENTS ON THE ACTION
OF STAPHYLOCOCCUS AUREUS ON THE
KLEBS-LOEFFLER BACILLUS.*

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Perhaps no subject is of greater interest both to the clinician and to the bacteriologist today than the treatment of disease carriers and their relation to epidemics. The dangers and the treatment of typhoid carriers have been the subject of most searching investigations during the last few years and still we are far from having solved the question. The diphtheria carrier has not been deemed worthy of so large a place in medical literature, but is a no less serious menace to society. The question how to rid the diphtheria convalescent's throat and nose of its virulent organisms is one of supreme interest to the physician. In communities in which strict quarantine is enforced the question of getting rid of the organisms and thus raising the quarantine is of no less interest to the patients. Diphtheria antitoxin has robbed this dread disease of much of its terror, but it seems to have no power to kill the causative organisms. Antiseptics are equally powerless, and in spite of all the resources of medicine, the quarantine is frequently prolonged for days, weeks, and even months after the membrane has disappeared and the clinical symptoms would proclaim the patient well. Yet the cultures and the microscope still show bacilli, typical in form and as malignant as in the worst phases of the disease.

Graham-Smith and Cobbett's[†] tables show that while the average period of persistence in virulent diphtheria cases is 31.6 days, about one-third of the cases exceeded this limit; six of these cases lasting over 100 days.

* Received for publication November 2, 1911.

† *The Bacteriology of Diphtheria*, edited by G. H. F. Nuttall and Graham Smith, 1908, p. 421.

In my own series of 175 cases, the duration was as follows:

10 cases terminated in 10 days or less.			
11	"	"	between 10 and 15 days.
40	"	"	15 " 20 "
25	"	"	20 " 25 "
35	"	"	25 " 30 "
33	"	"	30 " 35 "
7	"	"	35 " 40 "
6	"	"	40 " 45 "
2	"	"	45 " 50 "
1	"	"	50 " 60 "
2	"	"	60 " 70 "
3	"	"	70 " 80 "

From this table it may be seen that 63 per cent of the cases lasted less than 30 days, over 87 per cent of the cases lasted between 15 and 35 days, while only 12 per cent lasted under 15 days, and 12 per cent over 40 days.

It is no wonder, therefore, that the work published by Schiötz¹ in 1909, on the cure of chronic diphtheria bacillus carriers, attracted some attention. Schiötz noticed that a patient with staphylococcus sore throat failed to acquire the disease when exposed to diphtheria and also that convalescents from diphtheria in several cases ceased to give positive diphtheria cultures after an attack of ordinary staphylococcus sore throat. For these reasons he conceived the idea of treating slow convalescents with cultures of *Staphylococcus aureus*. His first patient, a young man, had been quarantined three months because cultures from his throat were constantly positive. Almost immediately after beginning the staphylococcus treatment the cultures became negative, and he was discharged on the fifth day.

The second patient, a woman of 55, cultures from whose throat had been positive for two months, gave negative cultures soon after beginning treatment and was released on the fifth day.

Four other cases were treated by Schiötz immediately after the clinical symptoms of the disease abated and with equally brilliant results.

Page² in 1911 reports one case in which he used the staphylococcus treatment. In Page's case, the clinical symptoms cleared

¹ *Ugesk. f. Laeger.*, 1909, 71, p. 1373; abstracted in *Jour. Am. M. Ass.*, 1910, 54, p. 422.

² *Arch. Inter. Med.*, 1911, 7, p. 16.

up in 14 days, but virulent Klebs-Loeffler bacilli were found in the throat cultures for about three months in spite of antitoxin, antiseptic sprays, etc.; 24 hours after beginning the staphylococcus treatment, only a few diphtheria bacilli were found, and the next day the cultures were negative and remained so for the following 15 days, during which the patient remained under observation.

Interested in these reports, I began a series of experiments to determine whether an antagonism existed between the *Staphylococcus aureus* and the Klebs-Loeffler bacillus which might account for these apparently marvelous results, or if not, whether any principles could be evolved which should place this treatment on a rational basis.

IN VITRO.

Fifteen strains of Klebs-Loeffler bacilli of different ages and varying degrees of virulence were isolated and planted on tube slants. Several methods were used to isolate the organisms, but two methods gave the best results: (1) About one part of human blood serum was added to 10 parts of plain or glycerin agar, inoculated, and poured on large plates and incubated. The colonies were then fished and transferred to serum tube slants. (2) The water of condensation of a serum tube was inoculated with a loop of mixed culture. Dilutions were made down through four or five tubes of serum. The tubes were laid down for several minutes in such a way that the slant surface was washed by the inoculated fluid. They were then placed upright in the incubator over night and the discrete colonies fished the next morning.

In the first seven sets, large serum tube slants and also blood-glycerin-agar tube slants were used; since it was found that the results in the two media corresponded exactly, only serum tubes were used in the last eight sets. Four tubes of each medium were used for each set. Tube 1 was inoculated with the diphtheria organisms alone. Tube 2 was spread with a mixture of about equal parts of staphylococcus and the diphtheria organism. Tube 3 was planted with the diphtheria culture and after 24 hours' incubation was spread with staphylococcus culture. Tube 4 was smeared with staphylococcus culture and after 24 hours' incubation the culture was killed by heating and a diphtheria

culture was then planted. This last tube was intended to show whether the staphylococcus in its growth produced any chemical substances or any chemical changes in the medium which would prevent the subsequent growth of the diphtheria bacillus. The results of these test-tube experiments were absolutely uniform and showed that in test-tubes at least there is no antagonism between these two organisms; that they grow well together, sometimes one and sometimes the other gaining a slight ascendancy, and that a killed staphylococcus culture forms a good medium for the growth of diphtheria bacilli.

IN VIVO.

In the following animal experiments various methods were used to test the action of the staphylococcus upon the Klebs-Loeffler bacillus and to determine whether either organism was antagonistic to the other when grown upon animal tissue. In the first eight sets, not much attention was paid to the taking of cultures during the life of the animal, the clinical symptoms and duration of life being the main criteria of the severity of the disease process. A brief account of these experiments follows:

Set 1. The culture was taken from a two-day case. Five guinea-pigs were used. No. 1 was injected in the groin with 0.5 c.c. of a 48-hour broth culture. Slight fever developed, lasting several days. There was swelling, heat, and tenderness at the point of inoculation. This broke and discharged on the sixth day, and after this the pig rapidly improved and was killed on the ninth day. No diphtheria organisms were recovered.

Guinea-pig 2 received an injection of 0.5 c.c. of a 48-hour broth diphtheria culture and 0.5 c.c. of a staphylococcus culture simultaneously. There was marked fever and local swelling and the pig died on the third day. Cultures from the site of inoculation showed only staphylococcus and no diphtheria bacilli.

Guinea-pig 3 received an injection of 0.5 c.c. of the diphtheria culture and 24 hours later the same amount of staphylococcus culture. This pig showed about the same symptoms as No. 1, and, like it, was killed on the ninth day. Staphylococcus only was recovered from the site of inoculation.

Guinea-pig 4 had its tongue scratched and swabbed with diphtheria culture. There was a slight illness and a membrane formed but the animal recovered in a few days.

Guinea-pig 5 received the same treatment as No. 4 and 24 hours later the infected area was swabbed with staphylococcus culture. This animal was sicker than No. 4 but apparently recovered after a few days. On the 20th day after inoculation, however, he suddenly died and diphtheria bacilli were recovered from lymph glands, and pharynx and larynx.

Set 2 was from a case of four weeks' duration. The five guinea-pigs of this set

were treated in the same way as those of set 1, except that in 4 and 5 a small area on the back was excoriated and swabbed instead of the tongue. All of the animals of this set were very sick and died before the end of the fourth day. The pigs receiving staphylococcus also were somewhat sicker and died sooner than those having only diphtheria cultures.

Set 3 was from a three-week case. Only two guinea-pigs were used. In both, a small area on the back was shaved and excoriated and swabbed with diphtheria culture. Twenty-four hours later guinea-pig 2 was swabbed with staphylococcus, the latter proceeding being repeated daily. No. 2 showed less fever and swelling than No. 1, but became much emaciated. No. 1 died on the fifth day and diphtheria bacilli were recovered from the wound. No. 2 was killed on the 14th day and no diphtheria bacilli were recovered, the culture being pure staphylococcus.

In set 4, which was derived from a six-week case, two rabbits were used, a small area on the back being excoriated and swabbed with the diphtheria culture. The next day rabbit 2 was swabbed with *Staphylococcus aureus* culture and each day thereafter. Neither rabbit showed severe symptoms and 10 days later the crust was removed and the backs reswabbed with the same cultures. Rabbit 2 died under the anesthetic. Rabbit 1 developed high fever and great swelling and edema and was treated with staphylococcus four days after the second inoculation and daily thereafter. He began to improve at once and 10 days after the second inoculation, cultures showed only staphylococcus, and the rabbit was discharged.

The culture used in set 5 was from a three-week case. Rabbits were again used and this time a clean, superficial incision was made in the back and four drops of broth culture introduced into the fresh cut. Rabbit 2 was treated daily with staphylococcus culture. There was fever and local swelling for about nine days in No. 1, and these symptoms lasted longer and were more severe in No. 2.

Set 6 was from a three-week case. Two rabbits were treated as in set 5. In both there was a slight fever and local induration around the incision. Both were about equally sick and for about the same time, but there was no growth on the last three cultures in No. 1, while in No. 2 staphylococcus developed on the cultures and there was still swelling and induration.

Set 7 was also a three-week case. Two rabbits were treated as in the last two sets. Rabbit 1 had a slight fever and swelling for a few days, but was healed and showed no growth on cultures by the 12th day and was therefore discharged. Rabbit 2, which received the staphylococcus treatment, had more fever and more marked local symptoms and also became much emaciated. It developed an abscess in the neck. Staphylococcus was still present in cultures from the wound on the 12th day.

Set 8 was also from a three-week case. Two guinea-pigs were inoculated in the same way as the rabbits in the last three sets. Guinea-pig 1 had very slight general and local symptoms, but diphtheria bacilli still persisted in the wound on the 20th day, although the pig had been apparently well for one week. Guinea-pig 2 showed about the same symptoms as No. 1 but became much emaciated. The diphtheria bacilli had disappeared by the 20th day.

In the next 12 sets daily or nearly daily cultures were taken in an effort to see whether the diphtheria bacilli disappeared from the wound any more quickly when treated daily with staphylococcus than when left untreated. In each of the 12 sets, three animals were used; a guinea-pig was injected with 2 c.c. of broth culture to test virulence and two rabbits were treated locally as follows: A small area on the

back was shaved, a superficial incision was made in the skin, and the skin was then dissected from the underlying tissue, thus making a pocket, perhaps one-quarter of an inch deep. This served to protect the inoculated area from contamination with hay bacilli and other organisms. Unfortunately many of the rabbits used in this series were afterward found to have been infected with another organism, which caused large foul abscesses and interfered to some extent with the cleanliness of the wound, and reliability of the results. In all of these rabbits, pockets were made as described and two loops from a serum tube were well mixed with the serum exuding from the tissues. Rabbit 2 of each set received daily treatments with a fresh broth culture of staphylococcus, and daily cultures were taken from both rabbits; after several cultures failed to show diphtheria bacilli, the animals were discharged as cured.

The first set of this series was from a two-day case. The guinea-pig died in 24 hours. Rabbit 1 had severe general and local symptoms and died on the fourth day, all cultures showing pure diphtheria bacilli. Rabbit 2 was also very sick at first, but improved after staphylococcus was used. The cultures changed in two days from pure diphtheria to pure staphylococcus and the rabbit recovered and was discharged on the 20th day, after numerous negative cultures.

Set 2 was a three-day case. The guinea-pig died on the fifth day and pure Klebs-Loeffler bacilli were recovered from the wound. Rabbit 1 also died on the fifth day and gave a pure diphtheria culture from wound. Rabbit 2 had much less severe symptoms and gave positive diphtheria cultures for four days. Afterward the cultures were always negative and it was discharged on the 16th day.

Set 3 was a 26-day case. The guinea-pig died on the sixth day. Rabbit 1 had slight general symptoms, but the local symptoms were pronounced and complicated by a *Staphylococcus albus* infection. The cultures were positive for diphtheria for five days. Rabbit 2 was much better than No. 1. Cultures were positive for diphtheria only three days. Afterward they were always negative and it was discharged on the 16th day.

Set 4 was a very virulent two-day strain and all the animals died within 48 hours. No difference was therefore noted.

In set 5 the guinea-pig was ill, but recovered. Rabbit 1 gave positive diphtheria cultures for six days. No. 2 had a much more marked local disturbance and retained the diphtheria bacilli until the 11th day. This rabbit was left with an abscess filled with white creamy pus.

In set 6 the guinea-pig died on the 18th day. Rabbit 1 had an infection and developed an abscess. The cultures from rabbit 1 ceased to show diphtheria bacilli after the third day while those of rabbit 2 never showed diphtheria bacilli.

Set 7 was from a 14-day case. The guinea-pig died in about 25 hours. Rabbit 2 died at the end of 48 hours, but staphylococcus only was recovered from the wound. Rabbit 1 died on the eighth day and no growth developed on the cultures.

In set 8 the guinea-pig died on the first day, while rabbit 2 died on the sixth day. Rabbit 1 showed but slight symptoms and was discharged on the 11th day.

In set 9 the guinea-pig died on the second day and both rabbits showed very severe symptoms, but recovered. Rabbit 1, however, developed an abscess. Cultures from No. 1 were positive for 10 days and those from No. 2 for eight days. The local condition in No. 2 was not so severe as in No. 1.

In set 10 the guinea-pig died on the second day and there was but little difference between the rabbits, as both had had a previous infection.

In set 11 the guinea-pig died on the first day and rabbit 2 died on the 10th day with diphtheria bacilli still present in the wound and in the heart blood. Rabbit 1 had very severe general and local symptoms and cultures were positive until the 10th day.

In set 12 both rabbits died on the second day, while the guinea-pig was discharged well on the 14th day. In the last series of four cases, the pocket inoculation method was used, but instead of the loops, a thin emulsion was made by mixing one small loop of culture with about 10 c.c. of sterile broth, and giving only a small amount, 1 c.c. being given to rabbits and two to three drops to guinea-pigs. Very little difference could be seen between those animals in which staphylococcus was used and those in which it was not. In three animals the diphtheria cultures remained positive longer in the animals treated with staphylococcus than in those not so treated, while in two cases, the treated animals became negative earlier than the untreated and in one case the time was the same.

All animals that died were examined as soon as possible after death; cultures were taken and tissues were fixed in Zenker's solution and sections cut. The tissue under the point of inoculation in all cases showed edema which was often hemorrhagic; sometimes inflammatory and degenerative changes were seen. One of the cases which died late showed granulation tissue.

The kidneys were affected in all cases except one. The changes varied from slight hyperemia to acute nephritis in some cases with hemorrhage. The adrenals in all cases were enlarged, dark red in color, and dripping with blood. Microscopic sections showed marked hyperemia throughout, severe hemorrhages especially in the central portion of the gland, often an outer narrow rim of gland tissue being the only normal portion of the gland. The cells in the central portion were usually more or less degenerated. The heart was examined in only a few cases. In those examined, a slight degree of fatty degeneration was found in five cases.

Of the 32 animals inoculated with diphtheria culture and afterward treated with staphylococcus culture, nine were apparently not influenced by the treatment, 14 were worse, and nine were better than the untreated diphtheria cases. Twenty-six and two-tenths per cent were therefore neither better nor worse than if they had received no staphylococcus culture, 41 per cent were in some ways worse, while only 32.5 per cent were better than the untreated diphtheria cases. Of the animals from which cultures were taken, however, the results are slightly different, inasmuch as 40 per cent retained the diphtheria bacilli a shorter time than the untreated cases, while 28 per cent retained them longer and 31 per cent for the same time.

So far, therefore, as these experiments go, there would seem to be no rational basis for treating diphtheria cases with *Staphylococcus aureus* culture. The two organisms grow together perfectly com-

fortably on artificial media. In the animal tissues while the diphtheria organisms frequently disappear more quickly in the treated than in the untreated animals, even this result is not to be relied upon and the clinical symptoms are often much more severe under the staphylococcus treatment. Moreover, in 28 per cent of the cases the diphtheria bacilli persisted longer in the treated than in the untreated animals.

It must be remembered, however, that it has been impossible in these experiments to reproduce at all exactly the conditions in the throat of a diphtheria convalescent, as these cases are all of necessity acute cases; both cultures are implanted on a raw, bleeding surface, instead of on a surface protected by an intact mucous membrane; and we have not in the skin a natural flora to assist us in our work. We must not, therefore, draw too stringent conclusions from the animal experiments alone.

I have been so fortunate as to be allowed to try the treatment on two human patients over whom it was possible to exercise almost perfect control. The histories of these cases are as follows:

1. A woman of 19 years.—August 1, 1911, she complained of sore throat of two days' duration. The symptoms were moderately severe; both tonsils were covered with membrane and the uvula was swollen.

The cervical lymph glands were enlarged and tender. Antitoxin was at first refused but 5,000 units were given August 3, i.e., on the fifth day of the disease. From this time all symptoms improved and the membrane grew smaller. Salicylates and benzoates were given internally and the throat was sprayed with phenol and iodine. Later lactic acid milk was used as a gargle. August 14 the throat was clean and the pulse and temperature normal. Cultures still continued positive for diphtheria, however, and August 25, Dr. Schlenker, who had charge of the case, consented to try treatment with staphylococcus culture. The only pure culture which I had at hand was a *Staphylococcus albus* from a case of chronic furunculosis. The day before beginning treatment the culture from the throat was a pure virulent diphtheria culture. The method used is that recommended by Schiötz and Page: A fresh staphylococcus culture in broth was made each morning, no culture over 12 hours old being used. The throat was well swabbed out with this culture by the physician. The rest of the culture was placed in an atomizer and the patient was told to spray her throat with it well every two hours during the day. A culture was taken each morning before beginning the day's treatment. August 26 and August 27, the cultures were negative. The treatment was then discontinued for 24 hours and the cultures taken the next two days showed a few diphtheria bacilli; because of the two negatives, however, the case was released from quarantine and no further cultures obtained until September 5; cultures taken on that date and also September 8 and 10 showed no

diphtheria bacilli. Many staphylococci appeared in the culture, but no local or general symptoms developed after the treatment. This case is not as conclusive either way as could be desired because of the reappearance of the Klebs-Loeffler bacilli after the two negatives had been secured and because five days were then allowed to elapse without treatment before the next negative was secured. The bacteriological history of this case is so interesting and suggestive that I append it here:

August	3—	Diphtheria bacilli (sick four days).		
"	8—	"	"	
"	12—	"	"	and many staphylococci.
"	13—	"	"	" " "
"	15—	"	"	few staphylococci.
"	16—	"	"	staphylococci equal.
"	17—	"	"	few staphylococci.
"	19—	"	"	many staphylococci.
"	21—	"	"	staphylococci equal.
"	21—	"	"	pure (another culture).
"	22—	"	"	nearly pure.
"	24—	"	"	" "
"	25—	"	"	" "
"	25—	Staphylococcus treatment begun.		
"	26—	Mostly staphylococci, no diphtheria bacilli, staphylococcus culture given.		
"	27—	Mostly staphylococci, no diphtheria bacilli, staphylococcus culture given.		
"	28—	Mostly staphylococci, no diphtheria bacilli.		
"	29—	Mostly staphylococci, a few diphtheria bacilli, staphylococcus culture given for last time.		
September	5—	Staphylococci and other organisms.		
"	8—	"	"	" "
"	10—	"	"	" " (sick 42 days).

2. Boy about 12 years of age.—He appeared at the hospital July 12, 1911, complaining of a sore throat which had lasted about four or five days. On examination, both tonsils were found tremendously swollen, so that the throat was almost occluded. The tonsils were covered by a thick yellowish exudate resembling pus. More posteriorly, however, a small patch of membrane could be seen and cultures were taken from this. Eleven thousand units of antitoxin were given in two days, after which the membrane disappeared. Potassium chlorate was given as a mouth wash and later buttermilk was also used. In spite of this, the cultures continued to be positive and on the 49th day of the disease, a pure diphtheria culture was obtained, 0.5 c.c. of which killed a rabbit on the fourth day. The staphylococcus treatment was therefore advised and begun September 2, the 58th day of the disease. The same method was used as in case 1. The cultures taken September 3 and 4 were still positive, but showed only a few diphtheria bacilli and on the fifth became negative. After four negatives had been secured the patient was discharged, but came back at intervals during the next two weeks to have cultures taken. All these cultures were negative. No injurious effects were noticed and indeed the patient said he felt much better after the treatment was begun. The staphylococcus used in this case was much more virulent than in case 1 and developed yellow pigment on the culture media.

The history of this case is as follows:

July	12—	Mostly staphylococci, a few diphtheria bacilli (sick five days).
"	14	" " " " " "
"	23	" " " " " "
August	3—	Staphylococci and diphtheria bacilli about equal.
"	8—	" " " " " "
"	9—	Staphylococci, few, mostly diphtheria bacilli.
"	12—	Diphtheria bacilli almost pure.
"	19—	Diphtheria bacilli and staphylococci about equal.
"	25—	Diphtheria bacilli, almost pure, few staphylococci.
"	29—	Diphtheria bacilli, pure.
September	2—	Staphylococcus treatment started.
"	3—	Staphylococcus mostly, a few diphtheria bacilli, staphylococcus culture given.
"	4—	Staphylococcus mostly, a few diphtheria bacilli.
"	5—	Staphylococcus mostly, staphylococcus culture given.
"	7—	Staphylococcus, no diphtheria bacilli, staphylococcus culture given.
"	8—	Staphylococcus, no diphtheria bacilli, treatment stopped.
"	9—	Staphylococci, no diphtheria bacilli.
"	11—	Culture negative and patient discharged (67th day).

Here again we have a staphylococcus throat on which diphtheria developed. The diphtheria bacilli, however, soon gained and then kept the ascendance.

So far as conclusions can be drawn from two cases, these two seem conclusive, were it not for the fact that quite frequently pure, positive cultures of long standing change to negative almost as abruptly without any treatment. Before definite conclusions can be drawn as to the value of this treatment many cases must be treated. During the coming winter, I hope to have opportunity to treat a large number of obstinate cases in this manner and a further report will then be made. Supposing, however, as seems probable from the seven cases previously reported and from my own two, that the staphylococcus may be depended on to clear up these chronic diphtheritic throats, what rational basis can be assigned for this action? Schiötz bases his claim on the failure of a staphylococcus throat to take the diphtheria infection when exposed and on the fact that some cases lost their diphtheria bacilli during a staphylococcus sore throat. This suggests a natural antipathy between these organisms. This theory seems untenable as in a series of 45 cases nearly every case began as a mixed staphylo-

coccus and diphtheria infection, and many of them were thus mixed throughout their entire course, while in the cases which became pure diphtheria culturally, the terminal cultures usually showed a gradual return of the staphylococci.

My test-tube experiments overthrow the idea of any chemical incompatibility.

It seems not to be a question of overgrowth of the area and crowding out of the diphtheria organisms, since in both the cases reported the terminal negative cultures gave but a light staphylococcus growth. The same objection applies to the inhibition explanation.

My own explanation of the action is based on cultures which I recently took from 12 normal throats, in all of which the staphylococcus was found the predominant organism, while in a few it occurred in pure or nearly pure culture. In nearly all the 45 cases of my series the first cultures showed mostly staphylococci with a few Klebs-Loeffler bacilli. In the more virulent cases the diphtheria bacillus gradually gained the ascendancy and finally was the only organism found, the others being possibly killed or inhibited by the antiseptics used to destroy the diphtheria organisms. Later, as the diphtheria bacillus lost its virility, the staphylococci gradually came back, regained the ascendancy, and finally were the only organisms found. In a certain few cases, however, the staphylococci were too few or the throat had taken on a reaction unfavorable to their growth and in these cases the growth of the friendly staphylococci might be favored by planting on the surface a young active culture.

SUMMARY AND CONCLUSIONS.

1. Test-tube experiments with 15 different strains of Klebs-Loeffler bacilli show that there is no inherent antagonism or incompatibility between *Staphylococcus aureus* and the Klebs-Loeffler bacillus.

2. Experiments on animals with 24 different strains of diphtheria organisms show that in 40 per cent of the cases the animals treated with *Staphylococcus aureus* got rid of their diphtheria organisms more quickly than did the untreated animals, while in 60 per

cent the cultures gave Klebs-Loeffler bacilli either the same time or longer. The clinical symptoms on the other hand were less severe in only 32.5 per cent of the animals treated with staphylococcus; as in the six patients treated by Schiötz and the one by Page, the throat culture in my cases which had been persistently positive for Klebs-Loeffler bacilli quickly became negative.

3. It would therefore seem advisable and effective to treat slowly convalescing diphtheria cases and diphtheria carriers with *Staphylococcus aureus* culture.

4. The animal experiments would indicate that a certain percentage of acute cases might clear up more quickly under the staphylococcus treatment than under the ordinary antiseptic treatment.

5. The animal experiments, however, also indicate that it would not be always altogether safe and frequently not successful to use the staphylococcus treatment before the mucous membrane of the throat has become intact so that it may protect the patient against invasion of the deeper tissues by the pyogenic organisms; hence it seems to me the treatment should not be used in acute cases, as Page advises, but only in those in which the diphtheria bacilli persist after the throat has healed and the patient is clinically well.

6. Two negative cultures are not always sufficient for a discharge, especially after this treatment, as the diphtheria bacilli may reappear after an apparent disappearance.

7. The reason for the apparently favorable action of *Staphylococcus aureus* on chronic diphtheria cases seems to be, not an antagonism or incompatibility between the two organisms, but an effort to reinforce the favorable, friendly throat flora, in the cases in which they are unable to regain their natural, normal ascendancy.

In conclusion I desire to express my gratitude to Dr. D. L. Harris for support and interest during the progress of this investigation and to Dr. Florence Evans for assistance, especially in the technical part of the work.

NOTE.—Just as this paper is going to press, a brief clinical report by Drs. Catlin, Scott, and Day appeared in the *Jour. Am. M. Ass.* of October 28, 1911. These physicians treated with *Staphylococcus aureus* eight contact cases, nurses who had diphtheria organisms in their throats after taking care of diphtheria patients, but who did not develop the disease. No harmful condition developed as a result of the introduction of the staphylococci, and cultures from the treated throats quickly became negative for diphtheria bacilli.

A CASE OF GENERALIZED INFECTION WITH A DIPHThEROID ORGANISM.*

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On account of our limited knowledge of diphtheria-like organisms and their effects and also because of the generally accepted custom of diagnosing diphtheria by the morphology and staining characteristics of its bacillus, the report of a case of non-diphtheric infection by an organism resembling the Klebs-Loeffler bacillus, morphologically and otherwise, must be of interest to bacteriologists. While the classical description of the Klebs-Loeffler bacillus gives us a clear and well-defined picture of a slender, curved, bipolar staining rod, tending to grow in irregular groups and often in parallel arrangement, we know that practically there is no organism which presents so varied a morphology. The short, rather thick, uniformly staining rod often seen at the beginning of the disease and also sometimes near its termination, the irregular, clubbed, involution forms, and the typical slender, bipolar forms give us a range of morphology which may well puzzle any but the most skilled and experienced diphtheriologists. And yet it is well known that, with all the bizarre forms met, there is something characteristic and convincing about the picture of a well-stained spread of Klebs-Loeffler bacilli. The various classes of pseudo-diphtheria and diphtheroid organisms, however, which are believed by some to be merely atypical or attenuated Klebs-Loeffler bacilli and by others to be new and distinct types of organism, still further complicate the subject and give a varied and constantly renewed interest to the study of the bacteriology and pathology of diphtheria. It is for these reasons that I venture to present the report of a case of local and general infection with an organism so closely resembling the Klebs-Loeffler bacillus that it was so diagnosed by a number of experienced diphtheriologists and yet differed from it widely in many of its cultural characteristics.

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The case which I wish to report is that of a girl, 16 years old, a housemaid who entered the Female Hospital of St. Louis, Missouri, with a diagnosis of lues. Her physical examination revealed a white girl of average development and good nutrition, with no deformities. The examination of the respiratory, circulatory, digestive, nervous, and urinary systems revealed nothing abnormal. The genital system showed no venereal sores and no signs of lues, but a slight leucorrhea was present, with no gonorrheal organisms in the discharge. On September 19, the patient suddenly developed a temperature of 104° , accompanied by nausea and vomiting, with enlargement and inflammation of the tonsils. The tonsils continued to enlarge until they practically occluded the fauces and almost prevented respiration. The diagnosis of phlegmonous tonsilitis was made and both tonsils were incised freely. The disease then ran a septic course with delirium, exacerbations and remissions of temperature, etc. Six thousand units of anti-diphtheric serum were administered September 20, but had no immediately favorable influence on the course of the disease. September 24, she developed a severe leucorrhea and three days later complained of pain about the vulva. On examination, an exudate was seen on the vulva giving the appearance of a membrane and the diagnosis of membranous vulvitis was made. The membrane soon disappeared, however, and the mucous surface showed only reddened, eroded ulcers, which bled readily on manipulation. The discharge from the vagina had at this time a very offensive odor. Patient also appeared deaf. Six thousand units of anti-diphtheric serum were again given, but again with no apparent influence on the course of the disease. On October 4, there was a profuse discharge from the right ear, having the same peculiar and offensive odor as that from the vagina. Later, both ears discharged profusely. On November 1 the vaginal lesion was apparently well, the throat completely healed, the hearing restored, and the patient feeling well, the only evidence of the condition being a slight discharge from both ears. For this clinical history, I am indebted to the kindness and courtesy of Dr. Brooks, assistant resident physician at the Female Hospital.

Bacteriology.—On September 19 a swab from the throat of the patient was spread on Loeffler's serum and sent to the City Bacteriologist for diagnosis. Only cocci and a few short bacilli were found and the report was therefore returned that *no* diphtheria bacilli had been found. On October 8 a culture was taken from the vaginal discharge and short slender bacilli were found in almost pure culture, so much resembling the Klebs-Loeffler organism both in morphology and arrangement and in their staining characteristics that a positive report of diphtheria was considered justifiable and such a report was sent with a request for subsequent cultures. Because of the curious and unusual course of the disease, numerous cultures were sent from time to time, and I began a systematic study of the organism, which was later found to be present not only in the vaginal discharge and in the vulvar exudate, but also in the discharge from the ear and throat, and in the main to the almost complete exclusion of other organisms.

The organism is a bacillus, somewhat shorter and of more regular form than the typical diphtheria bacillus, but very closely resembling it in morphology and staining characteristics. There is no tendency to the formation of threads, but the grouping is very similar to that of the Klebs-Loeffler organism. It stains readily with anilin dyes and reacts to Loeffler's methylene blue very much as does the diphtheria bacillus. With both Loeffler's methylene blue and with the Neisser stain, some of the organisms show distinct polar bodies, but most are either unipolar or stain uniformly throughout. With the Gram stain, the stain washes out, but with considerable difficulty, so that in

every preparation, many of the organisms retain the stain; for this reason it may well be regarded as gram doubtful. The organism is actively motile, especially in young broth cultures, and in this respect differs markedly both from the Klebs-Loeffler bacillus and from most of the diphtheroid organisms. It grows readily on most of the ordinary media, growing slowly at room temperature, while the optimum temperature is 37°. On Loeffler's serum, which seems the most favorable media for its cultivation, it forms a moist, soft, slimy, abundant, spreading growth, which is at first nearly colorless, but later becomes pale yellow and in a few days completely liquefies the serum. On potato, it forms a moist, elevated, rapidly spreading and abundant, almost colorless growth. On slant agar, it forms a slightly yellowish, moist, spreading growth with a tendency to soften and liquefy the agar. On blood agar, a small area of hemolysis surrounds a fairly large, elevated, yellowish, moist colony. On glucose agar, a very small amount of gas forms. In glucose broth, there is abundant gas formation, but none in lactose, maltose, or saccharose broth. All these sugar media gave an alkaline reaction after 72 hours. On plain broth there is a rapid growth, rendering the fluid at first cloudy, but later most of the growth settles to the bottom and sides as a granular, grayish-white precipitate. The gelatin stick culture rapidly liquefies the gelatin; an inverted cone is formed at first, but in 48 hours the entire tube is liquefied and most of the growth has settled to the bottom. In litmus milk there is slow acid formation and the milk is coagulated at the end of 72 hours. In all of the media, there is a characteristic, peculiar, sweetish, acetone-like odor, which is said to be similar to that noted in the patient. The 48-hour culture on peptone gives a pronounced indol reaction and a slight phenol reaction, but none for nitrites.

A guinea-pig injected intraperitoneally with 1 c.c. of a 48-hour broth culture showed no symptoms, but on repeating the injection a week later on the same pig, using 3.5 c.c. of a 24-hour culture, the pig died within about eight hours and the abdomen was distended with a slightly cloudy fluid in which the organisms were very abundant. Subcutaneous inoculations with both 24- and 48-hour cultures caused an induration at the point of inoculation; this increased in size and symptoms of illness, fever, weakness, etc., developed. Finally the indurated area softened and ruptured. The first pig rapidly recovered after the rupture of the abscess, therefore the second pig was killed soon after the breaking of the abscess and cultures were taken from the pus of the abscess, the peritoneal fluid, the heart blood, and the throat. No growth developed in the culture from the heart blood, but all the other cultures contained the characteristic organism and in nearly pure culture.

Forty-eight-hour broth cultures were filtered through a Reichel filter and the sterile filtrate inoculated intra-abdominally into guinea-pigs. These died in 36 to 48 hours and no organisms were recovered from the bodies. Hence the organism produces a toxin which is capable of killing guinea-pigs.

This organism differs from the Klebs-Loeffler bacillus then in the following particulars: (1) It is motile, while the diphtheria bacillus is non-motile. (2) It is somewhat less irregular in shape and staining than the typical Klebs-Loeffler bacillus and tends to grow shorter with age and to lose its polar staining, while the diphtheria bacillus tends to grow longer and to develop larger and more dis-

inct polar bodies and also bizarre involution forms. (3) Both organisms grow best on serum and at 37° C., but the diphtheria colonies are dry and granular, while the colonies in my case are moist and spreading and eventually liquefy the serum. (4) On agar, the differences are similar to those on serum. (5) Gelatin is never liquefied by the Klebs-Loeffler bacillus but is rapidly liquefied by the organism in my case. (6) The diphtheria bacillus tends to produce acid, while the organism I am describing produces an alkaline reaction in most media. (7) Diphtheria bacilli produce no gas in glucose broth, while mine produce abundance of gas in glucose broth, but none in lactose, maltose, or saccharose broth. (8) Milk is slowly coagulated by the organism in my case, while it is unchanged by the Klebs-Loeffler bacillus. (9) The diphtheria bacillus produces no indol, while mine produces an abundance of indol.

The organism I am describing was very pathogenic for the patient, but produced only a local abscess, with very slight general symptoms when injected subcutaneously into the guinea-pig; when injected intraperitoneally, it had no effect unless a considerable amount was used. As to contagion, while the patient was evidently able to reinfect herself, there is no history of others acquiring the infection from her. Her blood serum agglutinated the organism in dilutions of one to 50 and more slowly and less completely in dilutions of one to 100. A comparison of the morphological and cultural characteristics of this and a number of related organisms is given in the table which follows.

Proteus vulgaris has been inserted in the table because the odor noted in the patient and in most of the cultures, the liquefying action on serum, and the motility remind one somewhat of the proteus group. The morphology and arrangement, however, and most of the growth characteristics, as may be seen in the table, are quite different and place this organism indubitably in one of the many groups of diphtheroid organisms. Of all the diphtheria-like organisms which have been described, the one which my organism most resembles is the one called "*B. diphtheroides liquefaciens*" by Graham-Smith,¹ this being motile and liquefying serum. A similar

¹ *The Bacteriology of Diphtheria*, edited by G. H. F. Nuttall and Graham Smith, Cambridge, 1908.

	Organism in M. H.	Klebs- Loeffer	Hoffman	Xerosis	Graham-Smith B. Diph. Liquefaciens	Klein's B. Diphtheroid	Hamilton's Chromog. Bacillus	Ruediger Diph. Bacillus	Proteus Vulgaris	Colon Bacillus
Shape.	Short and slender	Long, slender, curved, irregular	Short, straight, oval. Median light band	Diphtheria-like, long, curved, and larger at ends	Long, slender, slightly curved	Diphtheria-like	Short, solid, granular rods	Regular, short, and long; granular	Short and plump or long and slender	Short, plump rod
Size.		1.5-3.5 μ long 0.4-1.0 μ diameter	1-1.5 μ long .12-.2 μ diameter						1-3 μ long $\frac{1}{3}$ -1 μ thick	
Stain (anilin dyes)	Deep and ready	Deep and ready	Deep, uniform, except light band	Ready	Ready	Diphtheria-like			Often irregular with light spot in center	Ready stain
Neisser stain	Often +	Bipolar +	Occasional	Positive	Positive	Positive	1. Negative 2. Positive	Neisser positive	Negative	
Gram stain	Doubtful	Positive		Positive	Positive	Positive			Actively motile	Motile
Motility	Actively motile	Non-motile	Non-motile	Non-motile	Motile	Segments			Chains	
Grouping	No chains, irregular grouping	Irregular grouping	Irregular grouping	Irregular	Irregular	Irregular	Irregular			
Involution forms.	Common and varied	Common and varied	May or may not be present	May be present	May be present	Present			Common	
Serum	Moist, grayish yellow. Liquefying	Grayish white, dry, and granular	Small, round, elevated, and pure white	Scaly, adherent colonies. Slow growth	Yellowish colonies; slow liquefaction	Granular colonies; liquefies	Abundant growth; liquefaction	Abundant growth	Often liquefied	
Agar streak	Grayish white, moist, spreading				Abundant, moist, yellowish growth	Slow growth	Greenish growth. 1. Agar purple 2. Purple growth	Abundant, many, moist, spreading		Uniform layer
Agar colonies.	Elevated, yellowish, moist, distinct	Large, translucent, with raised centers	Large, white, elevated, shining, moist, round	Small, gray, adherent					Stellate colonies	Grayish colonies

Gelatin.....	Cone-shaped depression, later complete liquefaction	No. liquefaction	No liquefaction	Little growth; no liquefaction	Liquefaction	Growth	Abundant	Liquefaction	Rapid growth, no liquefaction
Broth	Diffuse clouding	Whitish, granular pellicle. Snowstorm through fluid	Diffuse, cloudy, settling on sides	Clear, granular deposit	Cloudy, with granular deposit	Very little growth	Cloudy	Rapid, cloudy pellicle	Greenish, cloudy, rapid growth
Potato.....	Moist, colorless	Slight, dry, invisible	Opious, but invisible	Invisible growth	Abundant, white growth becoming yellow	No growth	1. Brown growth 2. Purple growth	Dirty, yellowish growth	Abundant
Litmus milk...	Slightly acid coagulation in 72 hours	Acid, but no coagulation	No acid and no coagulation	No acid and no coagulation	Coagulation	Coagulation	Turns white in 5-6 days	Initial acid and coagulum. Later proteolysis	Acid coagulation
Glucose broth.	Gas (much)	No gas	No gas	No gas	No gas	No gas	Acid	Gas	Gas
Lactose broth.	No gas	No gas	No gas	No gas	No gas	No gas		No gas	Gas
Maltose broth.	No gas	No gas	No gas	No gas	No gas	No gas		No gas	Gas
Saccharose broth.....	No gas	No gas	No gas	No gas	No gas	No gas		Gas	No gas
Optimum temperature.....	35°-37° C.	35°-37° C.	36°-37° C.					25°	37°-5° C.
Indol reaction.	Positive	Negative	None					Positive	Positive
Reaction of sugar media	Alkaline	Acid	Alkaline	Acid	Alkaline		Acid	Alkaline	Acid
Odor of cultures.....	Sweet, acetone-like odor	None characteristic						Putrefactive odor	Fetid odor
Virulence	Path. to patient; subcutaneous abscess in G. pig. No eff. intra-peritoneally except in large doses	Pathogenic	Non-pathogenic	Non pathogenic	Non-pathogenic	Produces subcutaneous abscesses	1. Non-pathogenic 2. Produces septicemia	Not virulent	Varies greatly

organism has been described by Hamilton, although it is difficult to say whether it is identical. The Graham-Smith bacillus, however, forms no gas in glucose broth and is non-pathogenic. In none of the diphtheroid organisms described have I found mention of a special odor, which is so characteristic and distinctive a feature of my organism. Hamilton, in a thorough study of pseudodiphtheria organisms found in cases of otitis media, attempts a classification of these organisms into the following three classes, basing her classification on their fermenting power. Her first class ferments dextrose and saccharose, but not maltose, lactose, or dextrin. It consists of short, uniformly staining rods, which are usually non-virulent. The second class ferments dextrose and maltose, but never saccharose. The organisms of this class closely resemble Klebs-Loeffler bacilli and are often virulent. The third class is typified by the Ruediger bacillus, which is a non-fermenting bacillus. The last-named bacillus she found but three times in an examination of 142 cases of otitis media, and the other two classes were represented in 51 cases. She concludes that these organisms are frequently the cause of otitis media. The fermenting power of these organisms makes a convenient basis of classification, although even on this basis it is difficult to include all of the diphtheroid organisms under any one of these three classes. My organism might be placed under her class 2, but for the fact that it does not ferment maltose. As to pathogenicity, the diphtheroid organism in my case, which was found practically in pure culture, appears to have been the cause not only of an otitis media, but also of tonsillitis and peritonsillar abscess and of an exudative vaginitis and vulvitis, together with fever, delirium, and other indications of a general intoxication.

THE FIXATION OF SOLUBLE ANTIGEN BY THE TISSUES.*

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(From the Department of Physiology of the University of Chicago.)

On the injection of an antigen into a susceptible animal a reaction takes place resulting in the formation of antibodies specific for the antigen injected; therefore a study of the seat of fixation of antigen is of importance in the study of antibody formation. Evidence has been presented showing that antibodies are formed (1) locally, that is, by the tissues at the site of inoculation of the antigen; (2) by the blood-forming organs; and (3) by the blood itself, particularly the leukocytes.

Hektoen and Carlson¹ injected dogs intravenously with rat and goat corpuscles—1 c.c. of a 10 per cent suspension per kilo—and three hours to six days after injection transfused the blood of such dogs into other dogs. They found no antibody formation in the recipients, but if the transfusion took place after the fourth day varying degrees of passive immunity were produced. A perfectly typical antibody production took place in the donors, showing that either sufficient antigen to cause an antibody production in the recipient was not free in the blood of the donor at the time of transfusion or the antigenic power of the injected corpuscles had been destroyed. These results support the view that antibodies are produced outside the blood stream. In these experiments the antigen—goat or rat corpuscles—was injected from three hours to four days before the transfusion. In performing the transfusion the recipient was bled dry, that is, until no more blood flowed from a severed carotid. The blood of the donor—the animal previously injected with antigen—was then pumped into the vessels of the recipient until the blood pressure in the recipient was equal to, or greater than, the pressure in the donor. However, in transfusing from the donor to the recipient until the pressure in both was equalized, it is doubtful if more than half of the blood of the donor

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¹ *Jour. Infec. Dis.*, 1910, 7, p. 319.

was transfused into the recipient, especially when most of the recipients used were smaller than the donors. The failure in antibody production in the recipients might be explained by the fact that sufficient antigen-containing blood was not transfused from the donor to recipient.

It is also possible that the rat or goat corpuscles, being morphological units of moderate size, may have been filtered out in the capillaries of the liver and spleen and retained mechanically, especially if agglutination occurred, or the endothelial cells acting phagocytically may have been instrumental in retaining the foreign cells within the body of the donor.

In order to determine more fully whether the retention of antigen in the body of the donor was a mechanical process or a true chemical fixation we decided to repeat the transfusion experiments of Hektoen and Carlson, using as an antigen some soluble substance that could not be filtered out mechanically or taken up phagocytically. To make certain that the major part of the blood was removed from the body of the dog injected with antigen, large recipients and small donors usually were used. The recipients were bled until no more blood flowed from the severed carotid and the blood of the donor was then pumped into the vessels of the recipient. As the recipient was larger than the donor, the donor could be bled until the pulse was scarcely perceptible. After the donor had been bled into the recipient until the pulse in the former was barely palpable a liter or more of normal salt solution was introduced into the vessels of the donor through the jugular or femoral vein. This restored the pulse for a time, "flushed out" the vessels of the donor, and made the exsanguination practically complete. The transfusion was continued until the pulse in the donor was again scarcely perceptible, and then stopped. The carotids of both the donor and recipient were ligated and the donor was restored by infusing normal defibrinated blood into the femoral or jugular vein.

We decided to use egg albumen or goat serum as the soluble antigen and the precipitin test as the indicator of antibody production.

Five dogs of about 10 K. weight were injected intravenously with 2, 4, 8, 20, and 35 c.c. of a 50 per cent solution of egg white. The dog injected with 20 c.c. died seven

days after the injection. Ten days after the injection the serum of the remaining four dogs was tested for precipitin, using egg white in dilution of 1/10, 1/100, and 1/1000 as antigen, and serum dilutions 0.1 to 0.001. All the serums tested were negative in dilution of 0.1 or more. A similar preliminary test was made, using undiluted goat serum as the antigen. One dog was injected intraperitoneally with 30 c.c. undiluted goat serum and three others intravenously with 12, 4, and 2 cc. of serum respectively. After ten days these dogs were tested with the following results:

Dog 1.—30 c.c. intraperitoneally; precipitin test positive to 0.01 c.c.

Dog 2.—12 c.c. intravenously; precipitin test positive to 0.1 c.c.

Dog 3.—4 c.c. intravenously; precipitin test positive to 0.1 c.c.

Dog 4.—2 c.c. intravenously; precipitin test negative in undiluted serum.

Dog 5.—Normal, precipitin test negative in undiluted serum.

Antigen was used in 1/10, 1/100, and 1/1000 dilutions in making the precipitin tests. Antigen in 1/10 dilution gave the best results.

Four other dogs of about 8 to 10 K. weight were injected intravenously with 6, 7, 7, and 8 c.c. goat serum respectively. Ten days later all showed a good precipitin test to 0.1 c.c. using goat serum in 1/10 dilution as antigen.

These findings show that goat serum injected intravenously into dogs in doses of more than 2 c.c. gives a good precipitin reaction in 10 days. After these preliminary tests a series of transfusion

No.	AMOUNT AND PLACE OF INJECTION	INTERVAL BETWEEN INJECTION AND TRANSFUSION	DAYS AFTER TRANSFUSION WHEN TEST WAS MADE	RESULTS (Antigen in dilution 1-10)	
				Donors	Recipients
1.....	8 c.c. intrav.	3½ hours	12	+	—
				(0.1 c.c.)	
2.....	8 c.c. intrav.	4½ hours	12	+	—
				(0.1 c.c.)	
3.....	8 c.c. intrav.	4¾ hours	12	+	—
				(0.1 c.c.)	
4.....	5 c.c. intrav.	3½ hours	9	+	—
				(0.1 c.c.)	
5.....	7 c.c. intrav.	3½ hours	9	—	—
				(0.05 c.c.)	
6.....	6 c.c. intrav.	3 hours	12	+	+
				(0.25 c.c.)	(0.25 c.c.)
7.....	6 c.c. intrav.	3 hours	12	+	—
				(0.25 c.c.)	
8.....	6 c.c. into heart	1 hour	12	+	—
				(0.25 c.c.)	
9.....	6 c.c. into heart	1 hour	12	+	—
				(0.25 c.c.)	
10.....	6 c.c. into heart	30 minutes	12	+	—
				(0.1 c.c.)	

+ = Precipitation.

— = No precipitation with undiluted serum.

The figures in parentheses give lowest active dilution of serum.

experiments were conducted to determine whether a soluble antigen was removed from the blood stream under the conditions outlined and the length of time necessary for the removal of a soluble antigen from the blood stream. That is to say, goat serum was injected intravenously into the donor and the blood of the donor transfused into the body of the recipient three, four, one, and one-half hours later in 10 sets of dogs (table on p. 45).

Transfusion technic.—In all cases the carotid of the donor was connected with the external jugular of the recipient using a T cannula of glass. Both donor and recipient were anesthetized with ether and placed on their backs, the left carotid and jugular of the donor and the right carotid and jugular of the recipient were isolated; a cannula was inserted into the proximal end of the jugular of the donor, and one into the proximal end of the carotid of the recipient, and each clamped off with a bulldog forceps. The distal end of the jugular in the donor and the distal end of the carotid in the recipient were ligated.

One arm of the T cannula was then inserted into the proximal end of the carotid of the donor and the distal end of the carotid ligated. The proximal portion of the severed carotid was clamped off with a bulldog forceps. The donor and recipient were then placed neck to neck and the other end of the cross-arm of the T cannula inserted into the proximal end of the jugular of the recipient. The distal end of the jugular in the recipient was ligated. The third arm of the T cannula was filled with salt solution, all air bubbles removed, and this arm clamped off with a bulldog forceps.

The recipient was then bled "dry," that is, until the flow of blood from the carotid ceased. (This blood was gathered in a sterile jar containing glass beads, and defibrinated.) The clamp on the proximal end of the carotid of the donor was then removed and the blood allowed to flow through the cannula from the carotid of the donor into the jugular of the recipient until the pulse in the donor had become scarcely palpable. Salt solution, usually about a liter, was then run into the proximal end of the jugular vein of the donor by gravity. This restored the pulse of the donor for a time and made the exsanguination much more complete than simple bleeding, the salt solution "flushing out" the vessels of the donor. When the blood was much lighter in color (as seen flowing through the glass connecting cannula) and much lowered in viscosity (as tested by allowing some to flow into a vessel from the third arm of the transfusion cannula), and the pulse of the donor was scarcely perceptible, the infusion of salt solution was stopped and the normal defibrinated blood from the recipient infused into the donor. This restored the pulse of the donor almost immediately. When the infusion of defibrinated blood into the donor was under way the connection of donor with recipient was severed, the vessels in the latter ligated, and the wound closed. When most of the defibrinated blood had been infused into the donor the cannulae were taken out of the vessels, the vessels ligated, and the wounds closed.

Small dogs were used as donors and large dogs as recipients. This made it possible to get over more blood, as there was more room in the vessels of the recipient for blood and blood diluted with salt solution. After ten or twelve days the serum of both recipients and donors was examined for precipitins using goat serum in dilution of 1/10 as precipitinogen.

Ten transfusions were made from 30 minutes to four and three-quarters hours after the injection of the soluble antigen—goat serum. The tests for antibody formation were made nine to 12 days after injection and transfusion. The results of this series of experiments are tabulated on p. 45.

An examination of the table shows that in each instance the donor gave a well marked precipitin reaction while the recipients failed to give any reaction in all but one case. This recipient gave a moderate reaction in the undiluted serum and a scant reaction in the 0.5 and 0.25 dilutions. The donor of this set gave strong reactions in the undiluted, 0.5 and 0.25 dilutions. This donor was the smallest one used.

From these experiments it is concluded that mechanical separation or phagocytosis is not a factor in the retention of the foreign corpuscles in the bodies of the donors in the series of transfusions performed by Hektoen and Carlson, and that soluble as well as insoluble antigens are fixed outside of the blood stream.

ON THE ANTIPNEUMOCOCCAL POWERS OF THE BLOOD IN PNEUMONIA.*

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Various observers—Wolf,¹ Boettscher,² Neufeld and Haendel,³ and Strouse⁴—report an increase in opsonins during lobar pneumonia, and the first observer found that after a preliminary fall the opsonins increase gradually until a maximum is reached shortly after the crisis. It is not possible to demonstrate this rise except with avirulent organisms, and as a result some doubt has been expressed as to whether this is really of importance in combating infection with the virulent and, at least in artificial conditions, non-phagocytatable pneumococci such as are isolated from actual cases of pneumonia.

Some investigators, indeed, have been unable to demonstrate any existence of protective antibodies of any sort in lobar pneumonia—among them Washbourn,⁵ and Seligmann and Klopstock.⁶ Their methods, however, were unsuited to show the presence of opsonins, and the fact that in their hands pneumonic serum failed to protect laboratory animals has apparently been satisfactorily explained by Neufeld and Haendel.⁷

The following work was undertaken to determine whether or not in cases of lobar pneumonia an increase of antibodies, and more especially of opsonins, could be demonstrated by the plate method. It was intended that this should be part of a more extended study; but as circumstances have arisen that make it impossible to continue the work at this time, the results so far obtained are presented here, although of a very fragmentary character.

The methods of investigation were as follows:

Pneumonic serum.—Specimens of serum, obtained with aseptic precautions, were separated from the clot and preserved in sealed tubes in the ice-box until the entire series from a given case of

* Received for publication November 20, 1911.

¹ *Jour. Infect. Dis.*, 1906, 3, p. 731.

² *Deut. Arch. f. klin. Med.*, 1910, 98, p. 93.

³ *Arch. a. d. k. Gsundtsamte*, 1910, 34, p. 166.

⁴ *Jour. Exper. Med.*, 1911, 14, p. 109.

⁵ *Jour. Path. and Bacteriol.*, 1895, 3, p. 214.

⁶ *Ztschr. f. Immunitätsf.*, 1910, 4, p. 103.

⁷ *Loc. cit.*

pneumonia had been obtained. Mixtures were then made up of equal quantities of serum, of a suspension of fresh, washed, normal human leukocytes, and of a very dilute suspension of a 24-hour growth of pneumococci on blood agar, a control mixture being made up with normal human serum. Immediately after mixing, equal small quantities measured by means of a capillary pipette were plated out on blood agar. The mixtures were then incubated three to four hours, and at the end of this time similar measured quantities were again plated out. After 18 to 24 hours' incubation, the number of colonies on each plate was counted.

Pneumonic leukocytes.—Specimens of serum were collected and treated as before. In addition, sterile blood was run into sodium citrate solution, and the corpuscles obtained in this way were washed immediately, along with a corresponding suspension of normal human corpuscles. The corpuscles were then stored in the ice-box over night; on the following morning they were separated from the supernatant salt solution by centrifugation, and counts were made both of the patients' and the normal leukocytes. One of the two suspensions was then diluted with its corresponding suspension of red blood cells, from which the leukocytic "cream" had been removed, to a degree sufficient to give the same number of leukocytes per unit of volume in both specimens. Then, with these suspensions, mixtures were made up exactly as before, using (1) normal human serum and normal leukocytes, (2) normal serum and patients' leukocytes, (3) patients' serum and patients' leukocytes, and (4) patients' serum and normal leukocytes. Plates were then made from these mixtures as before.

This method of study differs only in minor details from that previously used by Rosenow¹ and Tunncliffe.² Unfortunately a few attempts only were made to study the phagocytic activity of the leukocytes in this way. It was found difficult to run through an entire series of observations of the leukocytes in any one case, as, unless an extremely nice adjustment in the relationships between organisms and leukocytes is obtained, the results are obscured by either too many or too few organisms in the final mixtures.

The strains of pneumococcus used were two in number, both isolated shortly before from pneumonic sputum, and cultivated

¹ *Jour. Infect. Dis.*, 1910, 7, p. 429.

² *Ibid.*, 1911, 8, p. 307.

continuously on blood agar. The one used for the earlier work was of moderate virulence (contents of one agar slant produced death by pneumococcic septicemia in a guinea-pig in one week); the organism used later was somewhat more virulent, and as tested while the work was in progress was fatal to a white mouse inside of 12 hours with a dose of one-tenth of a blood agar slant.

The results follow, the figures in the tables indicating the number of colonies on the plates:

Case 1.—J. D., male, age 33. Typical right lower lobar pneumonia; delirium tremens. Seen first on eighth day. Crisis on ninth day. Uneventful recovery.

A. ACTION OF PNEUMONIC SERUM.

	At Once	1 Hour	2 Hours	4 Hours
Equal quantities of normal serum, normal leukocytes, and pneumococci.....	84	31	46	164
Equal quantities of patient's serum, 8th day, normal leukocytes, and pneumococci.....	86	21	5	4
Equal quantities of patient's serum, 9th day, normal leukocytes, and pneumococci.....	71	21	7	3
Equal quantities of patient's serum, 10th day, normal leukocytes, and pneumococci.....	84	26	7	1
Equal quantities of patient's serum, 15th day, normal leukocytes, and pneumococci.....	75	19	6	2

B. ACTION OF PNEUMONIC LEUKOCYTES.

	9TH DAY		15TH DAY	
	At Once	4 Hours	At Once	4 Hours
Equal parts of normal serum, standardized normal leukocytes, and pneumococci.....	450	8,100	810	20,000
Equal parts of normal serum, standardized pneumonic leukocytes, and pneumococci.....	500	4,700	790	4,700
Equal parts of patient's serum, standardized pneumonic leukocytes, and pneumococci.....	510	2,300	850	2,700
Equal parts of patient's serum, standardized normal leukocytes, and pneumococci.....	450	2,700	800	2,050

Case 2.—W. T., male, age 27. Lobar pneumonia involving entire left lung. Seen on sixth day. Crisis on ninth day. Persisting consolidation of upper lobe at time of discharge, 11 days later.

ACTION OF PATIENT'S LEUKOCYTES.

	6TH DAY		7TH DAY		10TH DAY	
	At Once	4 Hours	At Once	4 Hours	At Once	4 Hours
Equal parts normal serum, standardized normal leukocytes, and pneumococci.....	48	106	730	4,200	21	2,500
Equal parts normal serum, standardized patient's leukocytes, and pneumococci.....	63	92	975	3,700	26	2,500
Equal parts patient's serum, standardized patient's leukocytes, and pneumococci.....	48	9	820	350	12	2,300
Equal parts patient's serum, standardized normal leukocytes, and pneumococci.....	56	30	1,300	250	21	2,500

Case 3.—H. M., male, age 24. Right lower lobar pneumonia. Seen on eighth day. Crisis on 10th day. Uneventful recovery. See Chart 1.

ACTION OF PATIENT'S SERUM.

	At Once	2 Hours	4 Hours
Equal parts normal serum, normal leukocytes, and pneumococci...	130	890	About 9,000
Equal parts patient's serum, 8th day, normal leukocytes, and pneumococci...	119	970	About 9,000
Equal parts patient's serum, 9th day, normal leukocytes, and pneumococci...	153	640	About 3,000
Equal parts patient's serum, 10th day, normal leukocytes, and pneumococci...	149	118	608
Equal parts patient's serum, 11th day, normal leukocytes, and pneumococci...	149	60	66
Equal parts patient's serum, 12th day, normal leukocytes, and pneumococci...	170	60	52
Equal parts patient's serum, 13th day, normal leukocytes, and pneumococci...	147	100	58
Equal parts patient's serum, 14th day, normal leukocytes, and pneumococci...	181	43	23

Case 4.—P. L., male, age 37. Struck on chest by car four days before admission. On entrance a limited area of dulness, with bronchophony and crepitant rales, was found anteriorly over right lower lobe. Following this, entire right lung became consolidated. Apparent critical fall of temperature on seventh day, but with no apparent improvement otherwise. Death on 11th day. Autopsy showed resolving pneumonia of entire right lung, upper lobe less advanced than others. Large white kidney.

ACTION OF PATIENT'S SERUM.

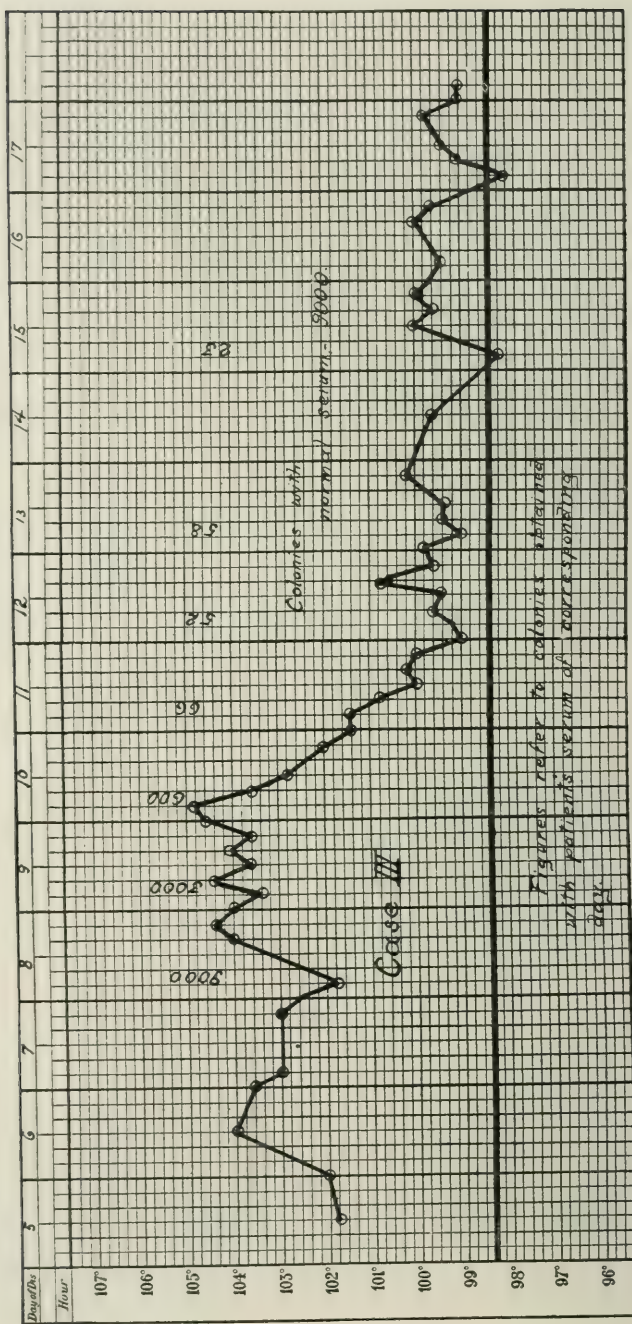
	At Once	4 Hours
Equal parts normal serum, normal leukocytes, and pneumococci...	104	780
Equal parts patient's serum, 7th day, normal leukocytes, and pneumococci...	137	1,310
Equal parts patient's serum, 8th day, normal leukocytes, and pneumococci...	111	1,300
Equal parts patient's serum, 9th day, normal leukocytes, and pneumococci...	99	1,870
Equal parts patient's serum, 10th day, normal leukocytes, and pneumococci...	106	1,620
Equal parts patient's serum, 11th day, normal leukocytes, and pneumococci...	124	720

One series of observations on this patient's leukocytes was also made; it failed to reveal any considerable difference from the normal in phagocytic activity.

Case 5.—C. L., male, age 58. Developed pneumonia while in hospital suffering from gastroenteritis. Seen on second day. Recovery by lysis.

ACTION OF PATIENT'S SERUM.

	At Once	4 Hours
Equal parts normal serum, normal leukocytes, and pneumococci...	51	93
Equal parts patient's serum, 2d day, normal leukocytes, and pneumococci...	47	141
Equal parts patient's serum, 3d day, normal leukocytes, and pneumococci...	61	289
Equal parts patient's serum, 4th day, normal leukocytes, and pneumococci...	64	152
Equal parts patient's serum, 9th day, normal leukocytes, and pneumococci...	53	40



ANTIPNEUMOCOCCAL POWERS OF BLOOD IN PNEUMONIA 53

Case 6.—W. W., male, age 31. Left lower lobe pneumonia. Seen on fourth day. Crisis on eighth day. Uneventful recovery.

ACTION OF PATIENT'S SERUM.

	At Once	4 Hours
Equal parts patient's serum, 4th day, normal leukocytes, and pneumococci.....	44	664
Equal parts patient's serum, 5th day, normal leukocytes, and pneumococci.....	39	522
Equal parts patient's serum, 6th day, normal leukocytes, and pneumococci.....	48	525
Equal parts patient's serum, 8th day, normal leukocytes, and pneumococci.....	40	112
Equal parts patient's serum, 11th day, normal leukocytes, and pneumococci.....	48	380

Case 7.—Mrs. S., age 53. Left lower lobe pneumonia. Seen on second day. Crisis fifth day. Uneventful recovery.

A. ACTION OF PATIENT'S SERUM.

	At Once	4 Hours
Equal parts normal serum, normal leukocytes, and pneumococci.....	30	630
Equal parts patient's serum, 2d day, normal leukocytes, and pneumococci.....	38	382
Equal parts patient's serum, 3d day, normal leukocytes, and pneumococci.....	30	332
Equal parts patient's serum, 4th day, normal leukocytes, and pneumococci.....	40	590
Equal parts patient's serum, 5th day, normal leukocytes, and pneumococci.....	57	155
Equal parts patient's serum, 6th day, normal leukocytes, and pneumococci.....	35	91
Equal parts patient's serum, 7th day, normal leukocytes, and pneumococci.....	31	332
Equal parts patient's serum, 9th day, normal leukocytes, and pneumococci.....	38	375
Equal parts patient's serum, 11th day, normal leukocytes, and pneumococci.....	33	500

B. ACTION OF PATIENT'S LEUKOCYTES.

	At Once	4 Hours
Equal parts normal serum, standardized leukocytes, and pneumococci.....	930	1,690
Equal parts normal serum, standardized patient's leukocytes, and pneumococci.....	990	2,150
Equal parts patient's serum, 4th day, standardized patient's leukocytes, and pneumococci.....	1,070	1,890
Equal parts patient's serum, 4th day, standardized normal leukocytes, and pneumococci.....	880	1,400

Case 8.—F. K., male, age 38. Bilateral lower lobar pneumonia. Seen on third day. Death on eighth day, without crisis.

A. ACTION OF PATIENT'S SERUM.

	At Once	4 Hours
Equal parts normal serum, normal leukocytes, and pneumococci.....	490	845
Equal parts patient's serum, 3d day, normal leukocytes, and pneumococci.....	382	500
Equal parts patient's serum, 4th day, normal leukocytes, and pneumococci.....	550	435
Equal parts patient's serum, 5th day, normal leukocytes, and pneumococci.....	430	551
Equal parts patient's serum, 6th day, normal leukocytes, and pneumococci.....	420	405
Equal parts patient's serum, 7th day, normal leukocytes, and pneumococci.....	435	580
Equal parts patient's serum, 8th day, normal leukocytes, and pneumococci.....	360	980

B. ACTION OF PATIENT'S LEUKOCYTES.

	3D DAY		4TH DAY	
	At Once	4 Hours	At Once	4 Hours
Equal parts normal serum, standardized normal leukocytes, and pneumococci.....	2,960	625	540	99
Equal parts normal serum, standardized patient's leukocytes, and pneumococci.....	3,190	845	550	2,600
Equal parts patient's serum, standardized patient's leukocytes, and pneumococci.....	2,900	435	430	330
Equal parts patient's serum, standardized normal leukocytes, and pneumococci.....	2,900	380	530	22

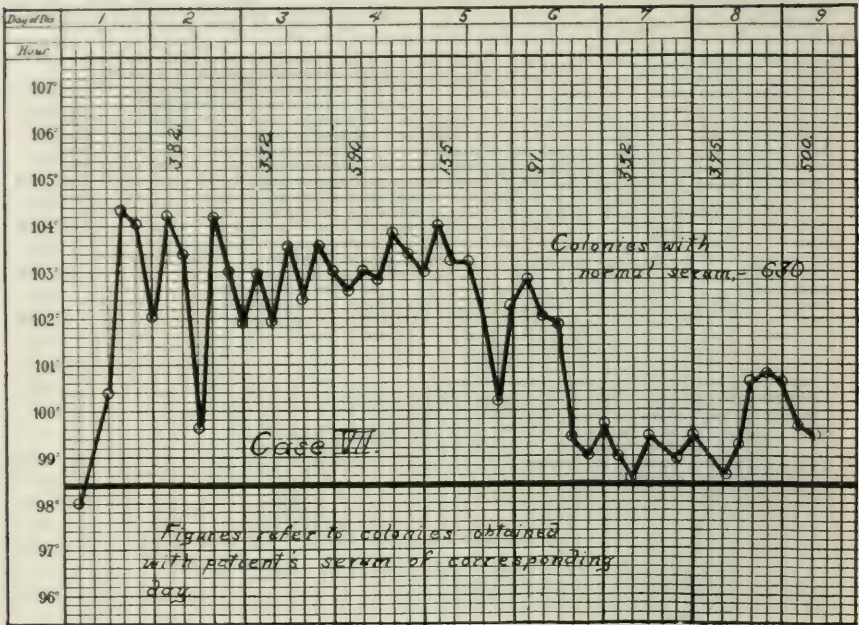


CHART 2.

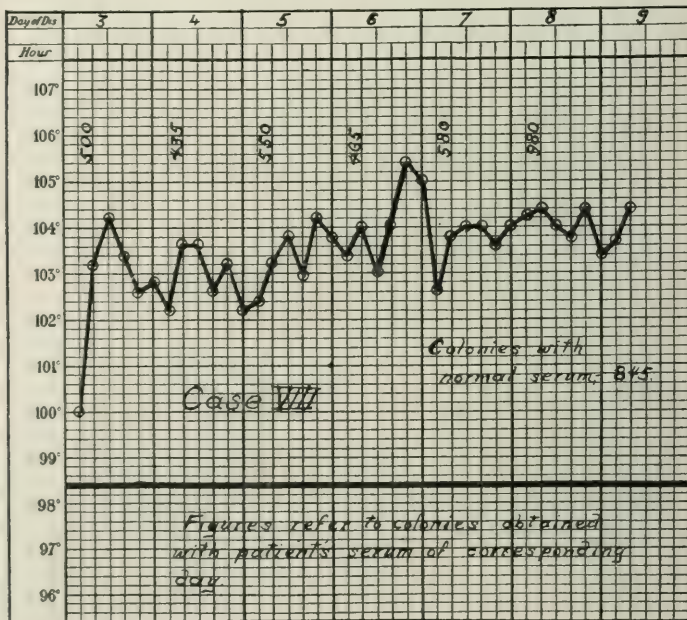


CHART 3.

From the foregoing results it would appear that in lobar pneumonia, as a rule, there is a development of antipneumococcal bodies usually progressing up to the crisis and reaching its maximum at or a little after this time. In two cases (4 and 5) this was absent, and in one (8) it was not well marked. But Case 4, although there was a crisis, if temperature alone be taken as an indication of this, showed no improvement at this time, and died not long after; so that the crisis at least was not typical. Case 5 recovered by lysis, and it is perhaps significant that late in the disease the serum in this case as well showed evidence of increased antipneumococcal power. Case 8, again, was one of exceptional severity; while at first there was evidence of antibody formation, this never became very marked, and disappeared entirely the day before death. Case 7 is peculiar in that there was little or no evidence of increase in antibodies until just before the crisis, which came on the fifth day, and that this increase was extremely transitory. The same transitory character of the increase in destructive power is shown also in Case 2, while in others again—1, 3, and 6—it persisted as long as the patient was kept under observation.

As to the nature of the particular antibodies concerned, the incomplete character of the experiments is not such as to elucidate this completely. Both agglutinins and opsonins have previously been demonstrated in pneumonic serum, and either of these might give results of the sort obtained and, especially, the opsonins. That these bodies, at least in part, are opsonins is indicated by the fact that the number of plate colonies was in some degree dependent on phagocytosis, as is shown by the dissimilar results frequently obtained when both normal and pneumonic leukocytes were used. An experiment, the result of which points in the same direction, is as follows:

	At Once	4 Hours
Normal serum, normal leukocytes, and pneumococci.....	450	6,000
Normal serum, salt solution, and pneumococci.....	490	200
Case 3, serum, salt solution, and pneumococci.....	480	92
Case 3, serum, normal leukocytes, and pneumococci.....	450	517

The great reduction in colonies in these mixtures in which serum alone was used is probably to be explained by the fact that the lack

of hemoglobin in them made them unfavorable media for the growth of pneumococci. But it will be observed that the relative disproportion between these mixtures in which leukocytes are present is much greater than in those in which serum alone was used. However, the fact that the colonies in the mixture with pneumonic serum alone were decidedly fewer than in that containing normal serum would indicate the presence as well of bodies not concerned in phagocytosis—presumably agglutinins.

In regard to the behavior of the leukocytes, unlike Rosenow,¹ who found in all of his cases heightened leukocytic activity, and in agreement with Tunnicliff,² who obtained instances both of increased and diminished phagocytic activity on the part of the leukocytes in pneumonia, the few cases studied here from this viewpoint showed examples of both modifications. Cases 1 and 2 gave evidence of greater phagocytic activity of the leukocytes than normal, and Cases 7 and 8 of the reverse. In these cases, it would be impossible to assert that the severity of the attack had any relation to this behavior of the leukocytes as was observed by Tunnicliff in her cases.

SUMMARY.

1. In most cases of lobar pneumonia there is obtained by the plate method distinct evidence of increased antipneumococcal power, as a rule, greatest at or just after the onset of crisis, and lasting for variable periods afterward.

2. The antibodies are apparently in part at least opsonins, and their activity can be manifested in proper circumstances even with organisms of some degree of virulence.

3. The cases in which this apparently characteristic increase of antipneumococcal power did not occur presented irregularities either in course or termination.

¹ *Jour. Infect. Dis.*, 1926, 3, p. 683.

² *Ibid.*, 1911, 8, p. 302.

SOME REMARKS UPON THE PUBLICATION OF PRESTON KYES ENTITLED "VENOM HEMOLYSIS."*

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AND

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In Vol. 7, No. 2, March, 1910, of this journal, Kyes has presented a summary of his earlier work on the subject of venom hemolysis with some new experiments, most of which are of the nature of repetitions. To him belongs the credit of recognizing that the snake venom hemolysis is brought about by the formation from lecithin, through the action of the venom, of the true hemolysin.

That the split products of lecithin should be hemolytic is, indeed, not extraordinary, but Kyes believes that in the reaction between cobra venom and lecithin something of a quite extraordinary nature takes place for which no analogy can be found either in chemistry or in the field of serology. The cobra venom is supposed to unite with the "lecithin-rest," and on the one hand to be a chemically definable substance, and on the other to possess the nature of the chemically indefinable toxins, inasmuch as it can give rise, in the animal body, to the production of antitoxin. The chemists who undertook the analysis of the new substance could, it is true, find only the lecithin-rest, while the toxins still defied chemical analytical methods. The only support, therefore, for an assumed toxine nature lay in the alleged property of the substance to stimulate the production of antitoxin.

In our previous publications[†] we were able to show that Kyes's view is incorrect. Antibodies cannot be produced against the so-called "lecithid," that is, against the derivative of lecithin which produces immediate hemolysis without incubation period. The experimental conditions under which Kyes worked were complicated

* Received for publication November 23, 1911.

† *Münch. Med. Wchnschr.*, 1908, 55, p. 105; *Biochem. Ztschr.*, 1908, 12, p. 407.

by the presence, in his preparation of "lecithid," of the hemolysin of native cobra venom, *and it was this, which upon injection into animals, caused the production of antitoxin, and against which alone the action of the antitoxin could be demonstrated.* The proof which we adduced against the toxin nature of the lecithin hemolysin is so convincing that further evidence would be entirely superfluous. Ehrlich and Sachs have also accepted our view and Sachs himself was never able to produce antitoxin by the injection of *heated* "lecithid."

In this connection, and in view of the statement of Kyes that the "lecithid" used by him for his immunization experiments had been boiled, it should be said that occasionally preparations of the lecithin derivative (which we have designated as "desoleo-lecithin") are obtained by Kyes's method in which, for some unknown reason, the native cobra hemolysin is resistant even to boiling.

EXPERIMENT.

From a 1 per cent watery solution of "desoleolecithin," prepared three weeks previously with freshly made ovo-lecithin, a further dilution 1-10 was made in physiological salt solution representing a final 1-1,000 concentration. A portion of this was used as control, a second portion boiled for one minute, and a third portion also boiled for one minute after the addition of an equal volume of $\frac{n}{10}$ acetic acid. After neutralization of the third portion and equalization of the volume in the three portions, it was found that the hemolytic power against washed ox corpuscles without the addition of lecithin was identical in all three; namely, $\frac{1}{10}$ c.c. of a 1-1,000 concentration.

With the addition of 0.5 c.c. of a 1-2,000 suspension of ovo-lecithin (1.0 c.c. of this suspension by itself was not hemolytic) the hemolytic power was equally increased in *all three portions* to $\frac{1}{20}$ c.c. of a 1-1,000 concentration, $\frac{1}{20}$ c.c. producing nearly complete hemolysis. These results are identical with those obtained by the examination made three weeks previously, during which period the preparation had been kept in the ice-box.

It is surprising, therefore, that Kyes, although he has not refuted our criticisms of his experiments, still clings to his disproven view. The new results which he presents in favor of the antigenic nature of "lecithid" are obtained by the very methods which we have shown to be fallacious. He again uses the antiserum after heating it to 64°-65° C., which seems to indicate that, without heating, the inhibiting effect could not be observed. That Kyes is in error, if he

still believes that his experiments are not controverted by our own, must be evident from the following citation:

"We convinced ourselves first of the correctness of Kyes's statement that a distinct difference is shown in the action of immune and normal sera when these have been heated for one-half hour at 64° C. It was found that heated normal rabbit's sera influenced the hemolytic activity of the lecithin derivative preparation in different degree, but that the inhibitory effect of the immune sera was still much more marked. The result was quite different, however, when 'lecithid,' serum, and blood were mixed at one time, and only that hemolysis taken into account which occurred within a few minutes. Under these conditions the heated immune sera protected exactly as did the heated normal sera. Since the lecithin derivative, in contrast with the native venom combined with lecithin, dissolves the corpuscles very quickly, and this is true of the smallest hemolytic dose, the result just described was very remarkable. It indicated that the specific antitoxin which was present in the immune sera but not in the normal sera was not directed at all against the lecithin derivative. Other circumstances also favored this view. It was further observed that the late hemolysis took place only on condition that *heated* normal serum was added. Fresh unheated serum did not impede the hemolysis at all. It was also noticed that the unheated immune sera did not cause any greater inhibition than normal sera and that the heated normal sera were exactly as antihemolytic as the unheated sera provided only that the quickly occurring hemolysis, which is characteristic of the action of the lecithin derivative, was taken into consideration. All of these observations were incompatible with Kyes's assumption that normal antitoxin is destroyed by heating. They showed very clearly that the heating did not eliminate an inhibiting property of the serum but gave to the serum a new quality—one that permitted hemolysis. The significance of this secondary hemolysis was easily recognized in its long period of incubation, which pointed clearly to a combination of lecithin with native cobra-venom. In the heated serum lecithin was present in *available* form, in which form it is not necessarily present in unheated serum; moreover, in the preparations of the lecithin derivative obtained by Kyes's method the active hemolytic constituent of the venom could quite conceivably be contained in solution. Our further experiments have established the correctness of this explanation in several ways.

"In the first place we were able to reproduce all the phenomena that accompany the use of heated sera by the addition of lecithin. Under this condition the same difference between normal and immune sera is made manifest.

"We found, furthermore, that, in the 'lecithid' preparation which we were using, the native venom constituent which is capable of acting upon lecithin was present in considerable quantity. Such small quantities of the preparation as were by themselves incapable of causing any solution of the corpuscles could produce complete hemolysis, after a period of incubation, upon the addition of lecithin."

In our second publication¹ we could demonstrate in this way about 40 per cent of the native hemolytic constituent unchanged in the chloroform lecithin layer, and in a recent experiment the freshly prepared "desoleolecithin" was found to contain as much as 60-70 per cent of the active ferment.

¹ *Loc. cit.*

"Finally, by heating the preparation in 1 per cent solution for three hours at 100° C. we succeeded in so altering it that it could no longer be 'activated' by the addition of lecithin. Under this treatment the rapid hemolytic power of the substance remained unchanged. By this means we obtained a 'lecithid,' the hemolytic activity of which was inhibited equally by immune and normal sera even when these were heated at 64° C. against this preparation. Also the inhibition of the heated hemolysin by heated normal sera corresponded with the inhibition of the unheated hemolysin by the unheated normal and immune sera. Every difference between the normal and the immune sera had disappeared.

"From all these facts it is evident that by the immunization antitoxin was produced, not against the lecithin derivative, but only against the native venom constituent contained in it."

Since the newer experiments of Kyes contain the same sources of error, the results are no more available as argument in this discussion than his earlier ones.

It is remarkable that Kyes pays no attention to this point upon which the entire argument rests, but contents himself with a criticism of the method with which we prepared the "desoleo-lecithin." We are satisfied that no importance attaches to the method of preparation, yet we would call attention to the fact that where the addition of alkali seemed necessary we have made it, and furthermore that the immunization of our rabbits was carried out with a preparation which had been made with the addition of alkali—as is specially mentioned in our publication.

We do not see the least reason in Kyes's article for changing our view, a further discussion of which is rendered superfluous by the more recent study of Manwaring,¹ who confirmed our results in every detail.

¹ *Ztschr. f. Immunitätsf.*, 1910, 6, p. 513.

FURTHER OBSERVATIONS ON A PLAGUE-LIKE DISEASE OF RODENTS WITH A PRELIMINARY NOTE ON THE CAUSATIVE AGENT, BACTERIUM TULARENSE.*

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In a previous paper¹ one of us described a disease found in nature in California ground squirrels (*Citellus beecheyi* Richardson) which is experimentally transmissible to other rodents, and which in practically all susceptible animals gives rise to plague-like lesions. The following is the summary of the observations reported in the paper mentioned:

A disease which presents lesions very similar to those of plague has been found among ground squirrels.

The disease is readily transmitted to guinea-pigs, mice, rabbits, monkeys, and gophers, and plague-like lesions are produced in at least some of these animals. Rats are but moderately susceptible to the infection. Cats, dogs, and pigeons appear to be immune.

The disease may be transmitted artificially by subcutaneous, cutaneous (vaccination), nasal, and intraperitoneal inoculation. The mode of transmission in nature is unknown, but there is some experimental evidence to suggest that fleas may serve as carriers. The disease is probably not spread by mere contact of healthy with infected animals.

The infectious agent has not been isolated. On account of the number of species susceptible, it seems likely that it is bacterial, not protozoan.

The causative agent is present in the circulating blood, as well as in the various tissues where it causes focal lesions.

The thermal death point of the organism as it is found in the animal's body is between 55° C. and 60° C.

Several observations appear to indicate that the disease is a febrile one.

We wish to report certain observations based upon a larger experience with naturally infected ground squirrels, and upon considerable experimental work, including the cultivation of the organism that causes the disease.

* Received for publication October 19, 1911.

¹ *Pub. Health Bull. No. 43*, U.S. Public Health and Marine Hospital Service, Washington, 1911.

THE DISEASE IN NATURALLY INFECTED SQUIRRELS.

Size and sex.—Of 42 squirrels found naturally infected with this disease 11 are noted as half grown, two as three-fourths grown, 25 as full grown, four size not stated. As to sex, 24 of these were males and 14 females; sex of four not stated.

Lesions.—A bubo is the most common lesion, though it is not constant. The affected gland is caseous and is frequently surrounded by a hemorrhagic area.

Spleen.—This organ is very much enlarged and is usually dark in color. A slaty appearance is not unusual. The essential lesions are whitish or yellowish caseous granules which vary in size from a pin point to perhaps 1 mm. in diameter.

Liver.—The only change noted in the liver is the presence of granules similar to those found in the spleen.

Lungs.—Lung lesions are rare. Granules have been noted in one case. In ground squirrels this rarity of lung lesions constitutes the most important anatomical difference between this disease and plague, since in the latter lung lesions are quite common.

FREQUENCY OF VARIOUS LESIONS.

BUBO:

Present.....	40
Absent.....	6
Single: inguinal.....	8
axillary.....	5
cervical.....	13
pelvic.....	6
Multiple: inguinal and pelvic.....	4
cervical and axillary.....	2
cervical and inguinal.....	2

SPLEEN LESIONS:

Present.....	35
Absent.....	11

LIVER LESIONS:

Present.....	21
Absent.....	25

LUNG LESIONS:

Present.....	1
Absent.....	45

Frequency.—The frequency with which the disease is encountered varies with the localities from which squirrels are received. Certain

districts show a high percentage of this infection, while it is absent in others. In a series of 10,000 squirrels coming from various points between the Sacramento River on the north and the Tehachapi Mountains on the south, this disease was observed seven times, while plague infection was found 10 times.

PATHOGENICITY.

The results of additional experiments to determine the susceptibility of certain animals previously used and of some that had not been made the subject of investigation are presented here.

*Susceptibility of rats (*Mus norvegicus*).* These rodents gave somewhat contradictory results in previous experiments; therefore we have made several observations on this subject. Large doses of infectious material given subcutaneously often result fatally. Small doses are less effective and inoculation by scarification usually fails to convey the disease to rats.

The following is a fair example of several experiments that we have performed: Six white rats were inoculated subcutaneously with a heavy emulsion made from the tissue of a guinea-pig dead of the disease. One died on the third day and two on the fifth day. They presented the lesions usually found in these rodents dead of this infection. The other three survived. Three white rats that were inoculated by the cutaneous method with the same material showed no evidence of illness.

Several experiments have been performed for the purpose of ascertaining whether there is any difference in the susceptibility of wild rats and of white ones. The most extensive one is presented here.

Series of rats, white and wild, were inoculated with various doses of emulsion made from the liver and spleen of a guinea-pig dead on the third day. Half a gram of each organ was ground in a mortar with sand and 10 c.c. of physiological salt solution. The emulsion was strained through cheesecloth and 1 c.c. given subcutaneously to each of two white and two wild rats. Each succeeding pair of each variety was given a dose one-tenth as large as that given to the preceding pair in the series. The results are set forth in the following table:

Two guinea-pigs were used for controls. One received 0.000001 c.c. and the other 0.0000001 c.c. of the emulsion. Both died of the plague-like disease on the eighth day.

From this experiment it seems that the two varieties of rats are about equally susceptible and that extremely small doses of the infectious material are occasionally successful in conveying the disease to these rodents.

WHITE RATS			WILD RATS		
Weight of Rats, Grams	Dose of Emulsion	Day of Death*	Weight of Rats, Grams	Dose of Emulsion	Day of Death*
160	1 c.c.	0	250	1 c.c.	neg.
160	1 c.c.	neg.	280	1 c.c.	neg.
130	0.1 c.c.	7	260	0.1 c.c.	6
135	0.1 c.c.	7	230	0.1 c.c.	9
100	0.01 c.c.	neg.	193	0.01 c.c.	neg.
115	0.01 c.c.	neg.	160	0.01 c.c.	neg.
105	0.001 c.c.	neg.	170	0.001 c.c.	neg.
130	0.001 c.c.	neg.	160	0.001 c.c.	neg.
135	0.0001 c.c.	neg.	215	0.0001 c.c.	neg.
115	0.0001 c.c.	neg.	150	0.0001 c.c.	neg.
140	0.00001 c.c.	neg.	145	0.00001 c.c.	neg.
115	0.00001 c.c.	neg.	140	0.00001 c.c.	neg.
100	0.000001 c.c.	neg.	120	0.000001 c.c.	9
70	0.000001 c.c.	neg.	60	0.000001 c.c.	neg.
90	0.0000001 c.c.	11	175	0.0000001 c.c.	neg.
95	0.0000001 c.c.	neg.	275	0.0000001 c.c.	neg.

* In each case where a rat died the diagnosis was verified by the inoculation of a guinea-pig with the spleen of the rat.

Lesions in rats.—The most constant and characteristic lesion of the plague-like disease in the white and the wild rat is a finely granular liver macroscopically indistinguishable from that so often observed in plague-infected rats. In addition there may be a fibrinous or necrotic area at the site of injection. The adjacent lymph nodes may be enlarged and congested but are not necrotic. The spleen is usually enlarged and dark, and occasionally granular. An intense subcutaneous injection may be observed. When the injection, the granular liver, and the large dark spleen coexist, the appearance is strikingly like plague.

Susceptibility of cats and dogs.—Two young dogs and four cats were inoculated subcutaneously with large doses of an emulsion made from the liver of a guinea-pig dead of this disease. The animals all remained well, while a control guinea-pig inoculated cutaneously with the same material died on the seventh day and

presented at autopsy the usual lesions. A white rat vaccinated with the emulsion remained well.

Susceptibility of the desert ground squirrel.—The desert ground squirrel (*Ammospermophilus leucurus*)¹ is a small rodent found widely distributed in the southern part of California. Two of these animals were vaccinated with spleen from a guinea-pig dead on the fifth day after inoculation from a naturally infected ground squirrel. One died on the fifth day, the other on the sixth. Both presented at autopsy bilateral axillary buboes, and granules in the liver and in the spleen. A ground squirrel (*Citellus beecheyi*) control died on the eighth day and presented the usual lesions of the plague-like disease.

Susceptibility of farm animals.—We have endeavored to determine the susceptibility of calves, sheep, swine, and goats. In the first experiment an emulsion was made by grinding in a mortar 1 gm. of spleen tissue from a guinea-pig dead of the disease (strain 1,059) and making a suspension in 10 c.c. of normal salt solution. One of each species was given 2 c.c. of the emulsion subcutaneously. A control guinea-pig inoculated by scarification with one loopful of the same material died on the sixth day with typical lesions. The calf, swine, and goat showed no effect from the inoculation. The sheep sickened, refused food, and died on the seventh day. At autopsy there was a bloody and somewhat gelatinous exudate at the site of injection and on the front of the thorax. The spleen was enlarged. A guinea-pig inoculated subcutaneously with a pea-sized fragment of the spleen died on the fourth day; a guinea-pig vaccinated from the spleen died on the seventh day; and one vaccinated from the gelatinous edema died on the sixth day. All had typical lesions.

To determine the persistence of the infectious agent in the bodies of the animals, material was aspirated from the site of inoculation on the days shown in the following table. A large hypodermic syringe was loaded with about 1 c.c. of physiological salt solution. This was injected into the tissue at the site, sucked back into the syringe, the needle withdrawn, and the material used to inoculate

¹ We are indebted to Mr. H. S. Swarth of the Department of Zoölogy, of the University of California, for the identification of this rodent.

a guinea-pig. The figures in the table show the day of death of the rodents. All died of the plague-like disease. The minus sign indicates that the test animals survived.

GUINEA-PIGS INOCULATED WITH MATERIAL ASPIRATED ON VARIOUS DAYS FROM FARM ANIMALS.

	1st Day 24 hours	2d Day	3d Day	4th Day	5th Day	7th Day	9th Day	12th Day	15th Day	19th Day
Calf.....	—	6	5	8	—	—	—	—	—	—
Swine.....	5	4	4	7	4	7	6	7	—	6
Sheep.....	5	—	7	4	5	Sheep dead	—	—	—	—
Goat.....	5	5	7	—	—	7	—	—	—	—

The results of the aspirations indicate that the calf carried the infectious agent until the fourth day, the pig until the 19th day, the goat until the seventh day, and the sheep until its death on the seventh day. Certain irregularities in the table probably indicate failure to reach the seat of infection with the needle used to withdraw the fluid.

In another series a salt solution emulsion was made of the liver, spleen, and bubo of a guinea-pig dead on the seventh day of the plague-like disease (strain 2,016). Four animals—a hog, a sheep, a goat, and a calf—received subcutaneously 2 c.c. of this suspension. A control guinea-pig which was inoculated cutaneously with the same material died on the seventh day with typical lesions. The hog and the sheep were negative. The goat developed an abscess at site. A guinea-pig inoculated subcutaneously with pus from the abscess developed a slough but recovered. The sheep died on the fourth day and presented the following lesions: Marked injection at site and a very small infiltration; a reddened, almost hemorrhagic area over front of sternum extending well down over the belly; below this an area of intense injection; a few black points on the surface of spleen; and considerable quantity of bloody, turbid fluid in pericardium. Guinea-pigs were inoculated by the cutaneous method with material from the site, from bubo, from spleen, and with heart's blood, respectively. The guinea-pig inoculated from the site died on the seventh day, presenting the usual lesions of this disease; the others survived.

To further test the susceptibility of sheep to this disease, three were inoculated. One was given a large dose and one a very small dose subcutaneously of emulsion of tissue of a guinea-pig (strain, 2.016), another was vaccinated with the spleen. The sheep that received the large dose sickened but recovered; the others remained well. The two guinea-pig controls that were inoculated subcutaneously with the emulsion died on the seventh and ninth days, respectively, with typical lesions.

NATURE OF CAUSATIVE AGENT.

Thermal death point.—The thermal death point had been found to be between 55°C . and 60°C . We have determined it within narrower limits than this. An emulsion was made of tissue (liver) from a guinea-pig dead on the sixth day after inoculation. Five-tenths of a c.c. of the suspension was placed in each of several drawn out glass pipettes. These were sealed at one end and immersed in a water bath for 10 minutes at the temperature shown in the following table. The heated material was then used to inoculate a guinea-pig. The maximum and minimum temperatures recorded by the thermometer during the 10 minutes that the emulsion was being heated are noted.

Range of Temperature $^{\circ}\text{C}$.	Fate of Guinea-Pig Inoculated with Heated Emulsion
53.8–54.2.....	Died sixth day; usual lesions
54.8–55.2.....	Died seventh day; usual lesions
55.8–56.2.....	Remained well
56.8–57.2.....	Remained well
57.8–58.0.....	Remained well

The results of this experiment indicate that heating to approximately 56°C .¹ for 10 minutes is sufficient to destroy the infective agent.

To determine whether there is a spore stage.—An emulsion of the tissue of a guinea-pig dead of the disease was mixed with enough sand to form a paste. This was permitted to stand in the laboratory exposed to the light for 48 hours. At the end of this period a suspension was made in saline solution of the sand-tissue mixture,

¹ We found that different thermometers of the same lot varied as much as 2°C . in their readings. The readings given here are those of the thermometer that read about midway between two others.

and 1 c.c. was injected into a guinea-pig. Another portion was heated in the water bath at 60°–70° C. for 10 minutes, and then injected into a guinea-pig. The animal that received the unheated suspension died of the plague-like disease on the fourth day. The one inoculated with the heated material remained well. We believe that this experiment justifies us in concluding that the organism as it exists in the tissues does not develop spores.

Filtration experiments.—Four Berkefeld filters were tested as to permeability by *B. coli*. Plates seeded with the filtrate from three of these remained sterile. Portions of an emulsion of organs of a guinea-pig dead of the plague-like disease were passed through each of these three filters and each filtrate was used to inoculate a guinea-pig subcutaneously. These animals remained well. The fourth filter permitted the fluid to pass through turbid and a guinea-pig inoculated with this material died on the fourth day with typical lesions.

Degree of septicemia.—In order to determine approximately the degree of septicemia in animals dead of the disease, we inoculated a series of guinea-pigs with varying doses of the heart's blood of a guinea-pig dead on the fifth day, and another series with the heart's blood of a squirrel dead on the eighth day after inoculation. In each case 0.0001 c.c. and 0.000001 c.c. reproduced the disease but 0.00000001 c.c. failed to convey the infection.

Relation to plague immunity.—On account of the close anatomical similarity to plague, it seemed worth while determining whether an immunity against plague protected against this disease. A guinea-pig which was inoculated subcutaneously on August 31, 1910, with bubo material from a case of human plague became very ill, but recovered. On November 27, 1910, this guinea-pig was vaccinated with a known highly virulent culture of the plague bacillus, but without any apparent effect. On February 3, 1911, it was inoculated subcutaneously with an enormous dose of a virulent culture of *B. pestis* but suffered no inconvenience. Twenty-two days later this animal was vaccinated with tissue from a ground squirrel dead of the plague-like disease. The guinea-pig died on the seventh day, presenting the usual lesions at autopsy.

TRANSMISSION IN NATURE.

The earlier work left much doubt as to the mode in which this disease is conveyed from squirrel to squirrel under natural conditions, and numerous additional experiments have not served to solve this problem definitely.

Feeding experiment.—It had been shown that the disease may be conveyed by feeding, and the following experiment appears to indicate that infection is very readily brought about in this manner. Two guinea-pigs and three squirrels were fed carrots mixed with the organs of a squirrel dead of the plague-like disease. Both of the guinea-pigs died on the fifth day. In each case there was a bubo in the neck and the other usual lesions. One of the guinea-pigs showed in the wall of the intestine numerous yellowish-white nodules varying in size from a pin point to 1 mm. in diameter. One of the ground squirrels died on the seventh day. A bubo was found at the base of the mesentery; none elsewhere. The usual lesions were found in the liver and in the spleen. The second squirrel died on the eighth day, and the third on the 19th day. Buboes were found in the neck of each rodent and the other characteristic appearances were present. In one there were large caseous masses at the base of the mesentery, and the wall of the intestine showed numerous small whitish nodules.

Lack of infectiveness of feces.—On two occasions we have taken fresh feces from cages in which guinea-pigs had died of the disease, made an emulsion of this material, and used it to inoculate guinea-pigs. In each case the result was negative.

Infection due to gross contamination of cages.—We have also failed to convey the infection through washings of the cage litter (feces, sawdust, etc.) from containers in which animals had been kept and had died of the disease. These results are in harmony with the fact that unless fleas were present we have never seen infection occur when healthy animals—ground squirrels or guinea-pigs—were placed in the cages with sick ones and kept there long after the death of the latter.

It seemed worth while determining whether heavy contamination of the cage with organs from a rodent dead of the disease

would lead to the infection of healthy animals. The spleen and liver of a guinea-pig, dead on the fourth day, were ground in a mortar, thoroughly mixed with sawdust, and placed in a cage with two ground squirrels. These animals both died, one on the eighth and the other on the 20th day. The lesions were typical in both rodents. Judged by the location of the bubo which in each case was in the neck, we consider it probable that the infection was transmitted by the ingestion of contaminated material. The presence of peripheral buboes in locations other than the neck leads us to believe that the disease is not contracted in nature¹ through feeding.

Flea transmission.—In the earlier paper, the infection of guinea-pigs with crushed fleas taken from rodents dead of the disease occurred only when the inoculations were made within 24 hours after the removal of the parasite from the host. We have made more extensive experiments both with squirrel fleas (*C. acutus*) and rat fleas (*C. fasciatus*) and have found that the insects may harbor the infection for a longer period. In each case fleas were placed in the cage with guinea-pigs inoculated with tissue from an animal dead of the disease and allowed to remain there until the animal died. As many fleas as possible were secured from the dead rodent and from the cage. Four of these were crushed and used to inoculate guinea-pigs at once after their removal from the dead host, and four (live) on three succeeding days. All of the rodents died that were inoculated with rat fleas immediately after they were taken from the dead guinea-pig. Two of those inoculated after 24 hours died, while only one of those inoculated at the end of 48 hours succumbed. The others remained well.

The experiment with squirrel fleas was carried out in the same manner. Two of the guinea-pigs inoculated at once with insects taken from the dead host perished, while none died that were inoculated on later days. Some of the guinea-pigs in this series developed what we regard as a chronic form of the disease.

In the preceding paper two cases were reported that might be regarded as probable examples of flea transmission. In each case ground squirrels infected with the disease were placed in cages

¹ See *Jour. Hyg.*, 1906, 6, p. 425, for the discussion of this subject in relation to plague.

with an abundance of squirrel fleas (*C. acutus*). Healthy squirrels placed in the cage with the sick developed the disease. It is obvious that such evidence is not conclusive but it is entitled to some weight.

We present here an experiment that proves conclusively that fleas may transmit the infection.

On January 21, 1911, a ground squirrel was vaccinated from the liver of a guinea-pig dead on the fifth day. The next day a large number of fleas (*C. acutus*) were placed in the cage with this squirrel. The rodent died on the seventh day and about 100 fleas were collected from the body. These fleas were placed in a clean cage with a healthy squirrel. The latter died 15 days later and presented the usual lesions of the plague-like disease, the bubo being in the neck. While there can be no doubt that this squirrel was infected by the fleas we are not sure that this is the usual means of transmission of the disease in nature.

We have made many attempts to carry the infection from one guinea-pig to another by means of squirrel fleas and rat fleas, but without success. We have also made a number of unsuccessful attempts to carry it by squirrel fleas from one squirrel to another. The failure of attempts to demonstrate the infection in feces and in cage washings, the failure of mere contact with infected rodents, the success of contact in the presence of fleas, and the one unimpeachable flea transmission experiment lead us to believe that these insects are concerned in the propagation of the disease.

A NOTE ON THE CAUSATIVE AGENT, *BACTERIUM TULARENSE*.¹

When smears from the spleen of a guinea-pig or a ground squirrel dead of this disease are stained with carbol fuchsin or aniline gentian violet, there will often be found large numbers of round or rod-shaped bodies usually lying inside well-defined, round, or oval clear areas. Several of these may occur within one clear space which for the sake of convenience will be termed the capsule. What appear to be these same rods minus the capsule are often found in the leukocytes. These objects, which we regard as the

¹ From Tulare, the county in California in which the disease was first observed.

essential cause of this disease, are very minute. The size is approximately as follows: Length of rod, $0.3\ \mu$ to $0.7\ \mu$, length of capsule $0.4\ \mu$ to $1\ \mu$; breadth of organism $0.2\ \mu$; breadth of capsule $0.3\ \mu$ to $0.5\ \mu$. With methylene blue this organism stains very poorly and shows no capsule. These encapsulated rods are found in enormous numbers in the spleen of nearly all guinea-pigs and squirrels, dead of this disease. The liver, bubo, and heart's blood usually contain the organisms, but in much smaller numbers than the spleen.

Attempts to cultivate the organism are not uniformly successful, and it is evident that we have not yet found the conditions best suited for its development. We have at present the seventh generation on Dorset's egg medium, the only culture material on which we have obtained a growth. A visible culture appears in three to five days in the form of transparent coalescent drop-like colonies. Guinea-pigs vaccinated with the first, second, and third generations died, presenting typical gross and microscopical appearances of the plague-like disease. Guinea-pigs vaccinated with the fourth and fifth generations developed a subacute form of the disease.

The morphology of the organism grown on artificial media is much like that described above. There is considerable apparent involution, enlargement, and irregularity of form. In some cultures large globular forms predominate. Unless the culture is mixed with something (serum) capable of giving a staining background the evidence of a capsule is usually not apparent. The microscopical appearance of smears from cultures as well as those from organs is very characteristic. In both cases the organism stains very poorly with methylene blue. It is apparently non-motile.

AN OUTBREAK OF TONSILITIS OR SEPTIC SORE THROAT IN EASTERN MASSACHUSETTS AND ITS RELATION TO AN INFECTED MILK SUPPLY.*†

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INTRODUCTION.

A sudden increase in cases of acute tonsilitis in certain parts of the city of Boston and its suburbs was talked of at a medical meeting in Boston on the evening of Thursday, May 11, 1911. By Sunday, May 14, the laboratories of the health departments were on the *qui vive*, for in many cases the disease simulated diphtheria, and in Boston, for example, there was an increase of 100 per cent in the cultures examined on May 14 as compared with the previous Sunday. In the Back Bay district of Boston, in Brookline and Cambridge, where the disease was most prevalent, individual physicians had from 20 or 30 to 60 or 70 cases of tonsilitis of a peculiar and characteristic type within a period of a week.

Certain physicians who were dealing immediately with the disease were quickly impressed with the fact that most of the families affected used a single milk supply, that from the Deerfoot Farms. The matter was brought to the attention of the officers and experts of the dairy company on May 13 and 14, and experts of the company as well as the inspectors of the Boston Health Department at once investigated conditions at the dairy and on the farms but without finding any evidence of a cause for the disease. Since the Deerfoot Farms Company had supplied milk in Boston for 28 years and in that time had been universally regarded as a pioneer in the work of dairy inspection and in the marketing of clean milk, many were loath to believe that it could possibly be involved. Physicians in other regions, and certain of the sanitary officials concerned, held that the Boston-Brookline-Cambridge outbreak was only part of a general excess of throat disease due to dust and extreme dry weather, and

* Received for publication November 11, 1911.

† Contribution from the Department of Public Health, American Museum of Natural History.

that the coincidence with a particular milk supply in the district affected was merely a local and accidental one. It was known that tonsillitis was prevalent in Hudson, where no Deerfoot milk is delivered, and also in Marlboro, where studies by Dr. W. W. Walcott of the State Department of Health showed no special incidence of disease among Deerfoot milk users. The uncertainty was so great that the local health departments concerned hesitated to take any definite position, in spite of the urgent requests of the company for advice and its willingness to take any steps that might be suggested.

About the middle of June, I was called in by Mr. Robert M. Burnett of the Deerfoot Company and asked to make a thorough study of the entire situation, and to make my findings public in such manner as I should see fit.

Tonsillitis is not a reportable disease and my records were obtained by conference with individual physicians, over 80 of whom were personally interviewed. Beginning my work so long after the event, there remain many lacunae which it has been impossible to fill. Nevertheless, I have been able, through the courtesy of these physicians, to obtain fairly complete records of 1,400 cases of the disease; and from a study of these records, in conjunction with other facts, it has been possible to reach a reasonably clear conclusion as to the general course and causation of the outbreak.

THE DISEASE.

A word should first be said about the disease itself. It was commonly called tonsillitis, but differed from ordinary tonsillitis in some respects, and was held by many physicians to be a new and peculiar pathological condition. The following picture is made up from conversation with a large number of the physicians who had treated it; and no one who has talked with many such can doubt that they have been dealing with a definite and specific entity. The disease is a variable one, and single cases may present hardly a feature in common with each other, but any series of 20 cases shows at once its characteristic features.

The onset of the attack was rapid and often accompanied by severe headache and acute grippy pains. The temperature was

high, often 10^3 – 10^5 . The throat was somewhat variable in appearance. At first there was a general diffuse redness extending over the tonsils and pharynx and adjacent regions, much like a scarlet fever throat. Later small isolated patches of white usually appeared, resembling the plugs of ordinary follicular tonsillitis. Still later in many cases there was an extensive membrane-like exudate simulating diphtheria. Frequently one tonsil was swollen first, followed by the other, and there was almost always more or less invasion of the cervical lymph nodes.

This first stage of the disease lasted for three to five days and was followed by recovery or by secondary complications. In either case the patient usually experienced great prostration. The commonest complications were abscesses in the peritonsillar region and in the cervical glands. The infecting organism, whatever it was, did not stay long in the throat, and affections of the nose and ear were relatively rare. It was the deeper tissues that were affected and abscesses produced were exceedingly painful and difficult to handle. Operations often failed to locate any pus, and after a time the abscesses discharged themselves, but the neck remained in a painful condition for a long period afterward.

In still more serious cases complications followed, of a more general septic nature. The occurrence of these acute complications was closely dependent upon the general condition of the patient. With children and young people the whole course of the disease was light; but with the old or those of low resistance, almost any weak organ might be attacked. Rheumatism was perhaps the commonest complication. Erysipelas was another. Nephritis occurred in a number of instances. Pleurisy and pneumonia in many cases followed the initial attack. The heart was frequently affected. One patient, Mrs. L., had, in succession, following her sore throat, erysipelas, pneumonia, and pericarditis. In the most dangerous cases of all, including many of the fatalities, there was a general systemic infection, leading to septicemia or peritonitis.

All these symptoms point to a streptococcus as the probable cause of infection, but there is as yet no definite information in regard to the bacteriology of the outbreak. Throat cultures examined at the Boston Board of Health Laboratory and elsewhere

showed no constant organism but Professor Theobald Smith, of the Harvard Medical School, has four cultures isolated from internal organs in the more severe cases, all of which are streptococci of apparently the same type. It is hoped that these may prove significant. I have no first-hand information on this point myself, as it was too late to do any bacteriological work when I was called into the case.

The disease described above is evidently something different from ordinary follicular tonsilitis, according to the opinion of all the physicians who have had most experience with it. So far as I am aware it has not before been noted in this country. Precisely similar outbreaks have occurred several times, however, in Great Britain, and in each case they have been traced to milk. The English have called such outbreaks "septic sore throat" and I have ventured to suggest this title as an alternative to the term tonsilitis which has been popularly applied to the present outbreak.

GEOGRAPHICAL DISTRIBUTION OF THE DISEASE.

Systematic inquiry soon made it clear that the distribution of the disease, as far as any abnormal excess was concerned, was far more restricted than rumor had suggested. Of course there is always some tonsilitis everywhere; and at any time certain localities may be expected to show more cases than normal, and many cities had a good deal of tonsilitis in the winter and spring of 1911. Such a condition as that which existed in Boston during the early part of May is, however, quite a different matter. When individual physicians have 50 cases within a week, and of a peculiarly severe type, and when the routine laboratory cultures of a large city show a sudden increase of 100 per cent, the condition is obvious to everyone and may properly be called epidemic.

Outside of Massachusetts I have been unable to find any abnormal epidemic prevalence of tonsilitis or any occurrence of peculiar septic throat disease during the winter and spring of 1911, with a single exception. There was a rumor that the disease had prevailed extensively in Washington and New York and was brought first to Massachusetts by a party of school teachers from Marlboro and Southboro who had been on an excursion to the former city. It

transpired, however, that this excursion was made long after the disease had broken out in the home towns of the excursionists and they presumably had carried the infection with them. Inquiries kindly made for me in Washington by Dr. W. F. Cuthbert among physicians of his acquaintance failed to show any excess of tonsilitis, and the local health department had no knowledge of any outbreak. Dr. R. W. Baker writes, "My recollection is that we had a large number of cases of tonsilitis in Washington last year—but we always have that." Dr. J. H. Bryan writes, "We had no epidemic of tonsilitis here, and there was nothing peculiar in the cases I saw from the usual form of this trouble that so frequently prevails in spring." Dr. A. B. Bennett reports no unusual prevalence of the disease. In New York again the local health department was not aware that anything unusual had occurred and certainly no tonsilitis epidemic was generally recognized among the physicians there.

Just outside New York City, however, in the town of Rye and adjacent parts of Westchester County, there appears to have been an outbreak of a peculiar type during the months of February and March. It prevailed for the most part among children, and there were no very severe complications, but Dr. Arthur S. Corwin, of Rye, who had 50 cases in his own practice, described to me the diffusely reddened throats, the diphtheria-like membranes, and the enlarged cervical glands which were so characteristic of the Massachusetts epidemic. I have no hint, however, of any connection between the two outbreaks.

As to the distribution of the disease within the state of Massachusetts, valuable information was courteously furnished to me by Dr. H. P. Walcott, chairman, and the other officials of the state board of health. As soon as the outbreak in Boston and Cambridge attracted attention the district medical inspectors were ordered to investigate the prevalence of the disease within their districts, and with the exception of two definite foci of infection, their reports were uniformly negative. The disease prevailed in epidemic form in Boston, Brookline, and Cambridge on the one hand, and in Hudson, Marlboro, and Southboro on the other; the rest of the state showed no abnormal conditions. In Worcester, where the newspapers reported 2,000 cases, a careful canvass of the physicians

showed nothing unusual, and Dr. J. C. Coffey, the health officer of the city, stated that the laboratory cultures showed no increase and that no outbreak came to his attention.

In view of the statements repeatedly made that there was much tonsillitis in the cities and towns of Framingham, Wellesley, Natick, and Newton, which lie along the Boston and Albany Railroad and connect the two principal centers of infection to which reference has been made, I made a special study of conditions in those communities. The western part of the town of Framingham shared in the epidemic of the adjoining districts of Southboro, and one physician, a member of the local health board, reports "quite a few cases." On the other hand, two other doctors, one of them the school physician, had noted no excess. In the adjoining town of Natick, to the east, five physicians were interviewed, including the school physician. All were agreed that there had been nothing abnormal here. In the Nathan Rice School there was a small outbreak of 10 or 12 cases about May 15; but they were light cases, of the ordinary follicular type. In the town of Wellesley the health officer, Mr. C. K. Blanchard, on hearing of the Boston outbreak, telephoned (May 26) to six physicians of the town, and found that there had been a slight excess of tonsillitis over the normal but nothing marked. There did occur, in the town of Wellesley, an interesting community outbreak which was simultaneous with the Boston epidemic but apparently in no way connected with it. In a Roman Catholic academy with about 125 children, 30 of the children and several teachers came down on May 8 all at once with a rather mild tonsillitis. The academy has its own milk supply, and for the most part its own food supplies, and so far as could be learned nothing had been brought in that could convey infection. Two of the sisters had quinsy sore throats in March and indications point to a local infection of some food supply within the institution, perhaps by an unrecognized carrier case.

In the city of Newton again there was no general excess of tonsillitis, except in the eastern part where it adjoins Watertown and Brookline and Brighton. Dr. F. G. Curtis, chairman of the board of health, and representative physicians in West Newton and Newtonville all testified to the absence of any general epidemic.

There was thus no connection between the two isolated foci centering at Boston and at Marlboro respectively. Inquiry into cases said to have occurred in other scattered localities, as at Scituate and Lowell, either gave no results or revealed a direct connection with the Boston outbreak.

The geographical distribution of the disease within the affected towns is also definite and worthy of notice. In Brookline the cases were confined chiefly to the Longwood district and to the region in the vicinity of Village Square. There was little or no disease beyond Harvard Street in the Chestnut Hill region. In Cambridge sickness was closely localized in North Cambridge and Old Cambridge, with little or none in the southeastern part of the city. In Boston the disease prevailed most extensively in the Back Bay and Allston, although cases occurred in South Boston, Dorchester, and Roxbury, with a few in East Boston. Charlestown and the North End with the adjoining cities of Chelsea, Revere, and Winthrop showed no excess.

It may be noted that all the districts specially affected were residential districts of a high class. The poorer portions of the city showed only a very slight response, such as might be expected on account of occasional contact through servants and in other obscure ways. Thus at the out-patient department of the Massachusetts General Hospital there were four new cases of acute tonsillitis treated during the first week of May, seven during the second week, 10 during the third, and three during the fourth. At the Boston Dispensary the corresponding figures were 3, 7, 10, and 4. In each case the outbreak in the second and third weeks was registered by a very slight increase.

EXTENT OF THE BOSTON-BROOKLINE-CAMBRIDGE EPIDEMIC.

With a non-reportable disease like tonsillitis it is of course impossible to gain an exact idea of the extent of an epidemic like that under consideration. In Brookline the local health department sent out two circulars to physicians, one on May 15 asking for a report of cases of tonsillitis since April 24, and another on May 25 asking for subsequent cases. These records were very courteously placed at my disposal, and from them and from interviews with a

few of the physicians I have compiled records of a total of 304 cases. This probably gives a fairly complete record of the outbreak, and not only so but it must include many cases of ordinary tonsillitis which would have arisen in the ordinary course of affairs in a community of 28,000 persons.

In Boston and Cambridge no official canvass was made, except that in Boston telephonic reports were obtained from physicians who sent in cultures for diagnosis. These were kindly placed at my disposal, but most of my cases were obtained by direct conference with the physicians themselves. In selecting physicians I naturally sought those who were reported to have had many cases and in this way I obtained mainly a record of the sharp outbreak, and not of the ordinary run of tonsillitis cases, of which the practitioners in other districts would normally have half a dozen during a month. Thus my Boston and Cambridge records represent the special outbreak, while the Brookline data include all tonsillitis of any type whatever.

In Cambridge the disease was sharply localized and was concentrated in the practice of a comparatively small number of physicians. I found 399 cases, but my canvass even here was very incomplete for Dr. E. A. Darling,¹ in a special study of the Cambridge outbreak, received from 35 physicians reports of 730 cases. In Boston the disease was scattered among a much larger number of physicians and my records represent a still smaller proportion of the total. In the Back Bay district I found 294 cases and in Allston 46 cases, but I made no canvass at all of South Boston, Roxbury, and Dorchester, where some cases occurred. The Boston Board of Health estimates 800 cases for the whole city and the figure is certainly a conservative one.

The total number of cases collected by me and used in my studies was therefore as follows: Boston (Back Bay), 294; Boston (Allston), 46; Brookline, 304; Cambridge, 399; total, 1,043.

EPIDEMIOLOGICAL CHARACTERS OF THE BOSTON-BROOKLINE-CAMBRIDGE EPIDEMIC.

Dates.—The distribution of the disease in time is indicated in Table 1 and in Fig. I. The dates given are, as usual in such

¹ *Boston Med. and Surg. Jour.*, 1911, 165, p. 904.

cases, those of the first visit of the physician, except in the case of a specialist called in for secondary complications, when the actual date of onset was estimated. In the late cases there had usually been a considerable period of sickness before the doctor was called in. In Brookline definite dates are lacking for many of those cases derived from the Health Department reports.

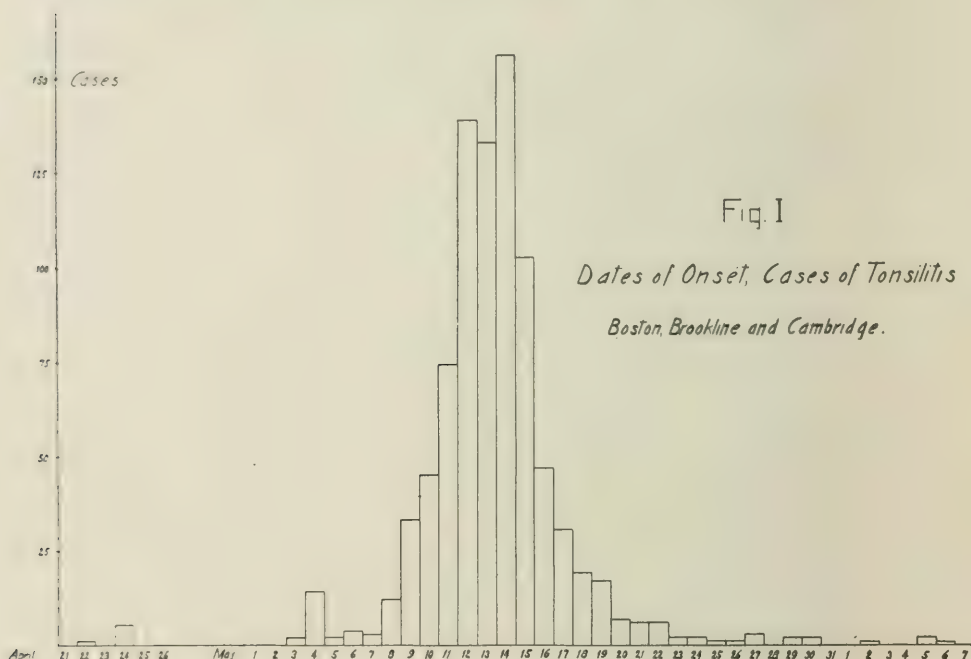
TABLE 1.
DATES OF ONSET, BOSTON-BROOKLINE-CAMBRIDGE EPIDEMIC.

DATE	DISTRICTS				
	Boston (Back Bay)	Boston (Allston)	Brookline	Cambridge	Total
April 22	1	1
24	3	..	2	..	5
May 3	1	1	2
4	0	..	1	1	14
5	1	1	2
6	3	1	4
7	1	..	1	1	3
8	3	..	3	6	12
9	17	1	0	6	33
10	15	..	12	10	46
11	18	11	12	33	74
12	48	8	10	64	130
13	52	..	20	52	133
14	61	6	10	72	158
15	27	3	27	46	103
16	12	2	10	23	47
17	6	7	2	10	31
18	1	3	..	15	19
19	2	..	2	13	17
20	1	1	1	4	7
21	1	..	1	4	6
22	2	..	6
23	1	1	2
24	2	..	2
25	1	1
26	1	..	1
27	3	..	3
28	1	1	2
30	2	2
June 2	1	1
5	1	..	1	..	2
6	1	..	1
Unknown	7	3	142	12	164

It is clear that we are dealing not with a diffuse prevalence of the disease but with a single (or perhaps double) sharply marked epidemic, culminating on the 12th, 13th, and 14th of May. The six April cases were most probably ordinary tonsillitis not connected with the main outbreak. I am inclined to believe, however, that the 14 cases on May 4 are significant, and indicate a very slight infection of the same general nature as that which caused the major outbreak. If so, it is an interesting illustration of a slight outbreak such as would never have been detected if the main epidemic had

not followed it, and such as probably does occur quite frequently without detection.

The major outbreak began on May 8 or May 9, quickly rose to a maximum, and again fell off, new cases having practically ceased by May 23. The curve points clearly to a single source of infection (except for the small outbreak of May 4) but perhaps to an infection extending over a period of several days. The evidence in half a



dozen cases where the time of infection can be fixed points to an incubation period of from two to three days, and the dates of the infection would on this hypothesis have been between the eighth and 11th of May.

The curve is so compact as almost to preclude the occurrence of any large number of secondary cases derived by contact from the primary outbreaks. Physicians were unanimous in holding that very few such secondary cases occurred. It may be concluded, therefore, that the disease was almost non-contagious in the form

and under the conditions in which it occurred in the neighborhood of Boston.

Family incidence.—The number of cases occurring in a household is shown in Table 2.

TABLE 2
FAMILY INCIDENCE, BOSTON-BROOKLINE-CAMBRIDGE EPIDEMIC.
NUMBER OF HOUSEHOLDS IN EACH CLASS

Locality	Number of Cases in a Household						
	1	2	3	4	5	6	7
Boston (Back Bay)*	80	20	16	6	4	5	
Boston (Allston)	7	8	3		1	1	
Brookline	101	31	17	0	2	5	2
Cambridge	107	53	20	12	8	4	2
Total	304	121	57	27	15	15	4

* 25 cases at an Art Students' Club.

The table indicates a heavy incidence upon the affected households. Of the families in which cases occurred 56 per cent had one case, 22 per cent two cases, 11 per cent three cases, and 11 per cent four cases or more. In view of the lack of evidence of secondary contact, these figures indicate that the common carrier of infection must have been a somewhat virulent one.

Sex incidence.—Data in regard to the sex of patients are given below in Table 3. They are somewhat incomplete, as certain physicians reported simply "cases" except for the name identifying

TABLE 3.
SEX INCIDENCE, BOSTON-BROOKLINE-CAMBRIDGE EPIDEMIC.

	Male	Female	Unknown
Boston (Back Bay)	42	157	95
Boston (Allston)	13	25	8
Brookline	66	90	130
Cambridge	94	241	64
Total	215	522	306

the household, and in the first part of the investigation no special effort was made to obtain fuller data. The records tabulated are sufficient, however, to bring out one striking fact—the very heavy incidence of the disease upon women. Twenty-nine per cent of my cases were males and 71 per cent females. It was thought at

first that this might be accounted for by the large number of cases among domestic servants, but an examination of the cards showed only 65 servants among the Cambridge and 62 among the Boston cases, not enough nearly to account for the discrepancy. It appears that there was some definite reason why females should be sufferers and reasons will later be brought forward for believing this to be less on account of greater susceptibility than on account of a greater exposure to infection.

Age incidence.—Data in regard to age distribution were not generally obtained but a special effort was made to collect them in the Cambridge canvass, as it was thought this city might serve as a type of the rest. The results are grouped by age periods in Table 4. For convenience the Cambridge deaths are grouped together with the cases, although the general subject of fatalities must receive special consideration by itself.

TABLE 4.
AGE INCIDENCE, CAMBRIDGE EPIDEMIC.

	Age Periods										
	0-5	6-15	16-25	26-35	36-45	46-55	56-65	66-75	76-85	86-95	Unknown
Cases.....	19	39	77	90	53	35	35	18	11	1	21
Deaths.....	1	0	11	0	1	4	4	5	5	1	0

The most significant thing about this table is the small proportion of children affected. Infants were almost free from the disease and even children of school age showed a comparatively small number of cases. Young adults between 16 and 45 included more than half the total. The gravity of the disease increased markedly with advancing years. Ninety cases between the ages of 26 and 35 did not include a single death, while after the age of 65 there were 37 per cent of fatalities and after the age of 75, 50 per cent.

Fatalities.—I have obtained, altogether, records of 48 fatal cases attributed to the Boston-Brookline-Cambridge outbreak of tonsillitis. There are considerable elements of uncertainty in the establishment of the relation between the original disease and its final sequel, because death often occurred from a complication of a somewhat remote kind. In some instances there was a rapid

septic invasion with no other symptoms. In most instances, however, the original throat attack was followed by pneumonia or by a heart attack or by some other affection, due partly to the weakening effect of the tonsilitis germ and partly to an original constitutional disability. Most of the deaths were among the old and weak, and the tonsilitis by itself would perhaps not have proved serious without these contributory causes.

The 48 deaths considered were distributed by places as follows: Boston (Back Bay), 18; Boston (Allston), 1; Brookline, 6; Cambridge, 23.¹ Seventeen were males and 31 females. The age distribution is indicated in Table 5.

TABLE 5.
AGE INCIDENCE, FATAL CASES, BOSTON-BROOKLINE-CAMBRIDGE EPIDEMIC.

	Age Period									
	0-5	6-15	16-25	26-35	36-45	46-55	56-65	66-75	76-85	86-95
Deaths	5	1	0	4	2	4	8	9	12	3

The table shows that with the exception of five deaths among infants and five among young adults fatalities were for the most part confined to ages above 45. Two-thirds of the deaths occurred at ages above 55, nearly half at ages above 65, and about one-third at ages above 75.

THE CAUSATIVE AGENT IN THE BOSTON-BROOKLINE-CAMBRIDGE EPIDEMIC.

There are three distinct possibilities to be considered in examining an outbreak of disease. The cause may lie, not in the spread of an infectious element, but in climatic or other general environmental conditions which in some way affect the human mechanism so as to favor the development of disease. Or an infection may spread from person to person by various irregular paths—by contact, by various foods, or perhaps by dust or the like, the path of infection being different in almost every case. This is known as prosodemic infection. Or, finally, the disease may occur in epidemic form, being spread at once to a large number of persons by a common source

¹ Dr. Darling in his investigation found 27 deaths in Cambridge.

of infection. All these three theories have had their adherents in connection with the outbreak under consideration.

The geographical distribution of the disease, in my judgment, negatives the first theory of general climatic influence. A dry spring, a large amount of dust, streets sprinkled with oil, and various other external physical conditions may or may not influence the spread of tonsillitis, but in any case there is no evidence that such conditions existed any more markedly in the affected districts than in others where there was no excess of tonsillitis. It was no drier and no more dusty in Cambridge than in Waltham, in Brookline than in Newton, in Boston than in Everett or Malden or Hyde Park. Clearly there was some definite source of infection in certain districts which was absent in others.

With regard to the view that tonsillitis was spread in prosodemic fashion, passing from person to person by diverse paths, the dates of the outbreak are practically conclusive. When disease spreads in this way, as tonsillitis and most minor nose and throat infections ordinarily spread, there is no special concentration in time. Cases straggle along for weeks and perhaps months. A sharp localization of a large number of cases, such as was so clearly manifest in the Boston-Brookline-Cambridge outbreak, points clearly to a single source of infection.

The simultaneous outbreak of epidemic tonsillitis on May 8 and the succeeding days in the town of Brookline and the cities of Boston and Cambridge, indicated graphically in Fig. II, must have been due to a common cause; and so far as I am aware, only two vehicles of infection—water and milk—have ever been found capable of producing such a phenomenon on so large a scale. It is quite inconceivable, even if dust ever spreads the germs of disease, that such a sudden and general infection should be due to this cause. Insects are not known to spread tonsillitis and are not prevalent in the vicinity of Boston in early May. Food supplies, like shell-fish, lettuce, and the like, are not distributed from any single common source to the large and widely separated districts under consideration. Probability pointed to one of the two more universal vehicles, water or milk, and since the water supplies of the three communities are distinct, more particularly to milk supply. A

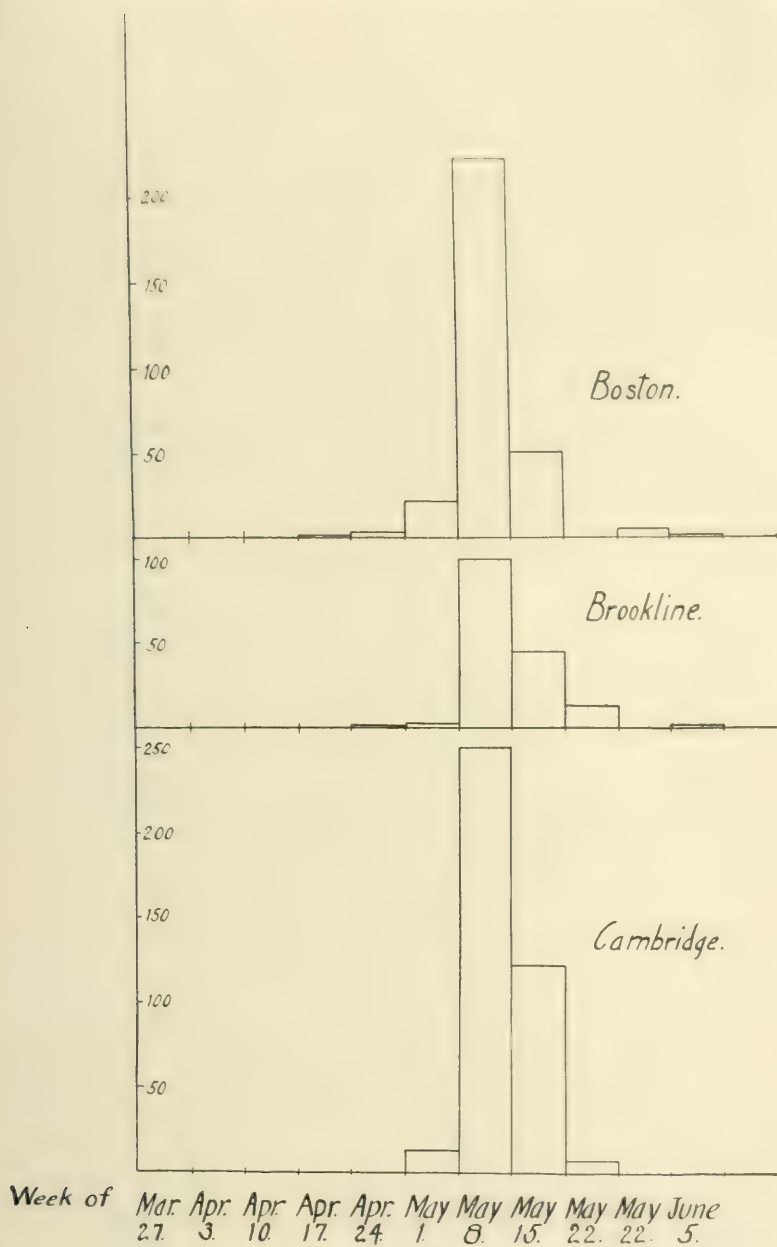


Fig. II.

*Weekly Incidence of Tonsillitis Cases:
Boston, Brookline, Cambridge.*

study of the relation between milk and the disease quickly showed this suspicion to be justified.

In the first place, it appeared that the general geographical distribution of the disease coincided closely with a single supply of milk. The Deerfoot Farms have two main supplies, one from Southboro and one from Northboro, and a supply of cream which is common to the two. It was the Southboro supply of milk alone which corresponded with the spread of tonsillitis. Wherever the Northboro supply went, no trouble occurred, a fact which explains why customers in certain large areas suffered no infection. Besides the car sent to Boston, milk was delivered direct from the Southboro dairy in Southboro and Marlboro. In both these places, as will be noted later, there was an outbreak of tonsillitis simultaneous with that in the neighborhood of Boston. Of the Southboro milk sent to Boston in the early part of May over 2,000 quarts were delivered in the Back Bay region. Here I have records of about 300 cases of tonsillitis, but as stated, these records are very incomplete and must represent at least 600 actual cases. Cambridge received nearly 1,000 quarts of milk and there I found about 400 cases. Brookline received about 600 quarts and there the complete board of health returns (including ordinary tonsillitis as well as the special epidemic) show 300 cases. In Allston with over 100 quarts of milk I found 46 cases. About 250 quarts of milk apiece were delivered to Roxbury and Dorchester, and 150 to South Boston. In none of these places did I make any systematic canvass, but in all it was ascertained by the Boston Board of Health that the disease occurred, and this fact was confirmed by local physicians interviewed by me. Wherever the Southboro milk went there was an excess of tonsillitis in the second and third weeks of May. In no district near Boston where it did not go was there any such excess, with one possible exception. In East Boston, it was reported to me by one physician with a large practice that he had three to 10 office cases of tonsillitis a day during the period of the general epidemic. Other physicians did not remember any such experience and it was impossible to get records of the specific cases. Skim milk from the Southboro dairy is sold in East Boston, and it is possible that this may explain the apparent anomaly.

On the whole, the general correspondence between Southboro milk and tonsilitis appears too close to be accidental. Furthermore, the parallelism extended to districts within the towns, as well as to the towns themselves. The various sections of Boston have already been considered separately, but the same phenomenon was apparent in Brookline and Cambridge. In the former town, tonsilitis prevailed in the Longwood district and near the old village, in the latter it occurred in North Cambridge and near Harvard Square, just where the Southboro milk is distributed.

The real test, of course, is the coincidence between milk supply and disease in the individual household; and on this point full data have been collected. In the first place, the physicians who gave me the cases were asked for any notes they might have made in regard to milk supply, and in the second place the names and addresses of the cases were carefully compared with the May 1 delivery lists of the Southboro milk, furnished to me by the Deerfoot Company. By this means the cases have been divided into three classes; those which appear on the milk lists; those which are believed by the physicians to have used the milk but do not appear on the lists; and those which are not known to have used the milk at all.

TABLE 6.
RELATION BETWEEN SOUTHBORO MILK AND TONSILITIS IN BOSTON AND CAMBRIDGE.

LOCALITY	CASES ON SOUTHBORO LIST		CASES SAID TO HAVE USED SOUTHBORO MILK BUT NOT ON LIST		CASES NOT KNOWN TO HAVE USED SOUTHBORO MILK	
	Number	Percentage	Number	Percentage	Number	Percentage
Boston (Back Bay)	251	85	22	8	21	7
Boston (Allston)	44	96	1	2	1	2
Cambridge	342	86	32	8	25	6

As far as Boston and Cambridge are concerned, over 85 per cent of the cases of tonsilitis appear on the Deerfoot Farm lists and 8 per cent more are believed by the physicians to have used the milk. In 11 out of the 32 cases in the doubtful class in Cambridge, the history of the use of milk at a friend's house or at work was quite definite and specific. It may be said that between 85 and 90 per cent of the cases of epidemic tonsilitis in Boston and Cambridge are

known to have used Southboro milk; and since this milk makes up less than 1 per cent of the total Boston supply and only a little over 2 per cent of the total of the Cambridge supply the evidence of a causative relation between the milk and the tonsilitis is irresistible.

TABLE 7.
RELATION BETWEEN SOUTHBORO MILK AND TONSILITIS IN BROOKLINE.

CASES ON SOUTHBORO LIST		CASES SAID TO HAVE USED SOUTHBORO MILK BUT NOT ON LIST		CASES NOT KNOWN TO HAVE USED SOUTHBORO MILK	
Number	Percentage	Number	Percentage	Number	Percentage
198	65	23	8	83	27

In Brookline, as indicated in Table 7, the results are somewhat different, because in Brookline the data include all cases of tonsilitis, and not merely the special epidemic. On such a basis as this it appears that something over 70 per cent of the cases were supplied with Deerfoot milk. A tabulation kindly furnished by the Brookline Board of Health, of cases in which the milk supply was definitely known, showed 214 cases among Deerfoot milk users and 56 distributed among 18 other dealers, the largest number on any one route being eight in one case and six in two others. The smaller percentage of Deerfoot cases in Brookline is precisely what should be expected, for the more ordinary disease is included, the less striking will be the relationship. In any large community there must always be a considerable amount of tonsilitis, irrespective of any special epidemic. This is shown in an even higher degree by the results of the house-to-house canvass carried out on May 26 under the direction of Dr. F. H. Osgood, veterinarian to the Brookline Board of Health. In this canvass all cases of sore throat were included, and 162 out of 474 houses supplied with Deerfoot milk were affected, against 161 houses out of 2,865 supplied from other sources. Thus about 50 per cent of all cases of sore throat occurred in the 12 per cent of the households canvassed which were supplied with Deerfoot milk.

These figures furnish unquestionable evidence that the excess of tonsilitis in the month of May was directly related to the distribution of the Southboro milk supply. Whether we consider that

90 per cent of the sharp epidemic in Boston and Cambridge or 70 per cent of all tonsilitis in Brookline or 50 per cent of all sore throat in Brookline was associated with a route supplying in Boston 1 per cent, in Cambridge 2 per cent, and in Brookline 7 per cent of the total milk, it is quite clear that no coincidence can account for such facts. Of the 329 households in Brookline on the Southboro delivery lists, 85, or 26 per cent, had cases of tonsilitis, and, of the 626 households in Cambridge, 154, or 25 per cent, were infected.

These general statistical conclusions are borne out by a mass of individual observations which, taken singly, might mean nothing, but together and in connection with the general facts are highly significant. A number of the more striking of these cases may be briefly cited.

At the S. Club, an art students' boarding-house, there were 25 cases, and the milk supply was from the Deerfoot Farm. At the S. Bank down town, there was an outbreak which on May 13 doubled the normal absences; the lunch-room used Deerfoot milk. In an apartment house in Cambridge, three families had Deerfoot milk and three did not; there were four cases in the first three families and none in the last. In an Allston apartment house, two families had Deerfoot milk and four did not; every member of the first two families (eight persons), except a baby with a special milk supply, had tonsilitis. In another family in Cambridge three adults who had Deerfoot milk had tonsilitis, and the children, with another supply, did not. In a Boston family, four milk drinkers suffered and the other two members did not. In this and many other instances the severity of the disease was proportional to the amount of milk consumed. In a physician's family in Boston, the husband and wife drank no milk, while three children and three maids used Deerfoot milk and all these last had tonsilitis. A housekeeper in Cambridge worked in Boston at a house where the milk was taken, and she and four of the Boston family came down. A woman at the North End worked in the family of a Back Bay physician where the milk was used, and came down at her own house. A child at Chestnut Hill drank Deerfoot milk at lunch on the Back Bay on May 12 and later suffered, although there was no other tonsilitis in the neighborhood. A relative of a Cambridge family drank the milk at their home once, and came down with tonsilitis at a summer place on Cape Cod, 48 hours later. Miss T. had no Deerfoot milk in Cambridge, but lunched in town with friends who used it, and she and they came down. Miss G. in Cambridge was a chronic invalid living in a darkened room and seeing no one. Her diet was cereals and Deerfoot milk and she developed the disease. Three cases of the same peculiar type noticed in the Boston outbreak occurred in the city of Lowell; and it appeared that Deerfoot milk was supplied to the family by special arrangement. Half a dozen cases like those mentioned above, where the date of infection can be rather closely placed, all point to an incubation period of 48-72 hours.

It will be remembered that the dates of onset (Fig. I) of the epidemic indicated a minor preliminary outbreak about May 4.

Of the 18 cases which occurred on May 3-5, 13 were known to have been supplied with Deerfoot milk. I am inclined to believe, therefore, that there was a slight infection of the milk before that date, distinct from the heavy infection which caused the major outbreak.

Of the 48 fatal cases included in my records, 29, or 60 per cent, appear on the Deerfoot lists, and 12, or 25 per cent more, are believed by the physicians to have used the milk, leaving 7, or 15 per cent, apparently unconnected with the milk outbreak. In some of these latter cases, the primary attack may probably have been ordinary tonsillitis.

The theory of milk-borne infection is in entire harmony with the geographical and chronological distribution of the disease. It explains the heavy family incidence, and it is in harmony with the marked excess of disease among females, since women, as a class, probably drink more milk than men. The comparatively small proportion of children affected appears, at first sight, to bear against milk infection. It must be remembered, however, that this is a disease in which vital resistance plays a large part, and testimony is universal that this particular outbreak, when it attacked children, usually appeared in mild form. That there were not more recorded cases among the young is probably, therefore, due to their high resistance rather than to their freedom from exposure.

THE OUTBREAK IN HUDSON, MARLBORO, AND SOUTHBORO.

So far nothing has been said about the second focus of infection, in the towns of Hudson, Marlboro, and Southboro, 20 miles to the west of the Boston district. The more or less simultaneous occurrence of an outbreak of tonsillitis in this region, to a large extent unconnected with Deerfoot milk, was one of the chief objections to the theory that the Boston outbreak was milk-borne; and the objection at first appeared to be a serious one.

Inquiry among physicians in the three towns named soon showed that there had, indeed, been an outbreak of acute tonsillitis in all of them, and furthermore that the disease had been of exactly the same peculiar type observed in Boston. The physician's description of the disease, and its complications, tallied exactly with those given in Boston and Cambridge. The general diffuse redness of

many of the throats, the occasional diphtheria-like membranes, the high temperature and grippy pains, the large number of peritonissillar abscesses and cervical glands, the abscesses often showing little or no pus when opened, the recurrences, the resulting rheumatism, pneumonia, erysipelas, and nephritis were all noted. As in Boston, the disease was light with children and severe with older persons, but there were fewer very acute cases and no general septicemias. One death, probably attributable to the outbreak, occurred in Hudson, a man in middle life in whom the tonsilitis caused a sudden recrudescence of a chronic myocarditis.

Records were obtained altogether of 392 cases—97 in Hudson, 169 in Marlboro, 62 in the village of Southboro, and 64 in the two large Southboro boarding-schools. Excluding the school cases, the general epidemiological characters may be briefly considered and compared with the Boston data.

The household incidence is indicated in Table 8 and it is at once evident that the concentration of cases is much less than in the Boston outbreak. In the latter 56 per cent of the households had only one case and 22 per cent had three or more. In the Marlboro district over 83 per cent of the households had single cases only, and only 6 per cent had three or more.

TABLE 8.
FAMILY INCIDENCE, HUDSON-MARLBORO-SOUTHBORO OUTBREAK.
NUMBER OF HOUSEHOLDS IN EACH CLASS.

Town	Number of Cases in a Household			
	1	2	3	4
Hudson.....	59	8	6	1
Marlboro.....	124	13	5	1
Southboro.....	36	8	2	1
Total.....	219	29	13	3

The sex incidence, as shown in Table 9, was markedly different from that of the disease in the Boston region. In Boston, Brookline, and Cambridge the incidence was more than twice as heavy on females as on males; in Hudson, Marlboro, and Southboro males were affected most, 55 per cent of my cases being males and 45 per cent females.

Data in regard to age were obtained in Hudson, and are tabulated in Table 10. They correspond pretty closely with the Cam-

TABLE 9.
SEX INCIDENCE, HUDSON-MARLBORO-SOUTHBORO OUTBREAK.

	Male	Female	Unknown
Hudson.....	44	45	8
Marlboro.....	76	57	36
Southboro.....	27	18	17
Total.....	147	120	61

bridge data (Table 4), with the single exception that there were fewer cases among old people. I have no record of a single case over 65 years in Hudson, while 30 Cambridge cases were above that age.

TABLE 10.
AGE INCIDENCE, HUDSON OUTBREAK.

	Age Periods							
	0-5	6-15	16-25	26-35	36-45	46-55	56-65	Unknown
Cases.....	5	18	10	23	17	10	4	10

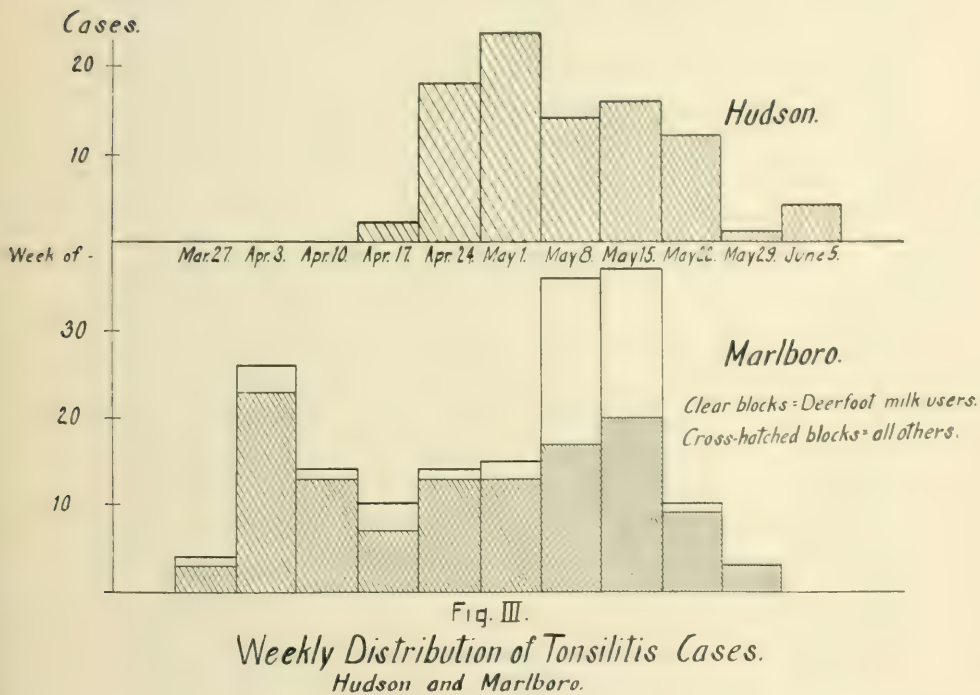
The real key to the Hudson-Marlboro-Southboro situation lies in the remaining factor, the distribution of the disease in time. The general facts are summarized in Table 11 for all three towns, but they can best be discussed separately, taking Hudson first as the simplest case.

TABLE 11.
DATES OF ONSET, HUDSON-MARLBORO-SOUTHBORO OUTBREAK.

DATE, WEEK BEGINNING	NUMBER OF CASES IN		
	Hudson	Marlboro	Southboro
March 27.....	..	4	1
April 3.....	..	26	5
10.....	..	14	2
17.....	2	10	..
24.....	18	14	3
May 1.....	24	15	5
8.....	14	36	15
15.....	16	37	11
22.....	12	10	20
29.....	1	3	..
June 5.....	4

In Hudson there is no Deerfoot milk delivered, and the fact that tonsillitis occurred there appeared at first sight to throw doubt on

the importance of milk as a causative factor in Boston. The distribution of cases shows at once, however, that we are dealing with a totally different condition from that obtaining in the Boston-Brookline-Cambridge epidemic. The Hudson figures are plotted in the upper half of Fig. III and the Boston, Brookline, and Cambridge figures for the corresponding weeks (but on a smaller vertical scale) in Fig. II. In Boston there was an explosive outbreak due



clearly to a single cause. In Hudson there was a gradual spread of the disease extending over a period of five weeks or more. The fact that other neighboring towns did not have the disease, while Hudson did, shows that there was specific infection and no effect of general climatic conditions; but a mere inspection of the dates of onset is sufficient to negative the idea of any common vehicle of infection localized in time. I was unable to find (by inquiry among the physicians) any common bond of location or association or food

supply between the cases. The infection must have spread in what is known as prosodemic fashion, as commonly occurs when influenza or diphtheria or ordinary colds spread through the community, not being carried by any single common vehicle like water or milk, but passing from one to another, by direct contact, by handling fruit or lead pencils or other objects that go into the mouth, and in a hundred different ways. Prosodemic disease always shows the slow, straggling development that appeared in the Hudson tonsillitis. It does not exhibit the marked concentration in families, characteristic of a milk outbreak, and it is more likely to affect young persons than the old, and men than women, on account of their more varied and widely ranging life.

Conditions in Marlboro were not quite so simple, but can be understood by a brief examination of the facts. Deerfoot milk, to the amount of about 800 quarts a day, was sold in Marlboro but the investigation made by Dr. W. W. Walcott, district inspector of the state board of health, showed that out of 40 affected families canvassed, only 18 had used this milk. A survey of the dates of onset of my 169 Marlboro cases (Table 11) shows that, as in Hudson, no single sudden infection could be expected, since the disease prevailed for at least eight weeks, practically through both months of April and May. The disease was confined to no one milk supply and no one section of the town, but spread gradually and by diverse routes through the whole community. Clearly this, as in Hudson, must have been for the most part a case of ordinary prosodemic infection.

On the other hand, it will be noted that the two weeks between May 8 and May 22 showed a particularly heavy incidence of tonsillitis, 36 and 37 cases, against 15 and 26 for the next highest weeks. These are precisely the weeks in which the milk epidemic occurred in Boston, Brookline, and Cambridge. The idea is naturally suggested by these facts that in Marlboro we are dealing with a two-fold phenomenon, a prosodemic prevalence of the disease extending over the whole two months, and a milk epidemic coincident with that in Boston. I have compared the names of my cases with the Deerfoot delivery list, and the results plotted in the lower curve of Fig. III show that the conclusion is a correct one. Of the 96 cases in

Marlboro before and after the weeks of May 8 and May 15, only 12, or 12 per cent, were Deerfoot customers; of the 73 cases in the fortnight while the Boston outbreak was going on, 36, or 49 per cent, were Deerfoot customers. The cases among non-Deerfoot milk users (shown in cross-hatching) remained about as they had been; while the Deerfoot users (shown by the clear outlines) exhibited a marked increase. The tonsillitis which had been existing all through April and May in Marlboro was increased during these two weeks by the presence of milk infection, but the major part of the outbreak, like that in Hudson, was of other and, apparently, prosodemic origin.

In Southboro conditions were essentially the same. Tonsillitis of the peculiar type in question existed all through the months of April and May, but was increased after May 8 by milk-borne cases. Notably, the two boarding-schools in the town suffered from a clear-cut Deerfoot milk epidemic of 42 cases among the 169 boys at St. Marks and 17 cases among the 70 boys at the Fay School. These figures are not included in the previous tabulations, which include only the 62 cases reported from the village of Southboro. In the adjoining town of Westboro there were a few cases of the prosodemic type, five, of which I have records, occurring in the month of April.

Two questions suggest themselves in connection with a comparison of the two outbreaks which I cannot answer altogether satisfactorily. The first is why there was not a more severe epidemic of milk-borne tonsillitis in Marlboro between May 8 and May 22. If the milk had been as heavily infected as in Cambridge there should have been several hundred cases, yet I found records of only 36 cases among Deerfoot users during this fortnight, after interviewing almost all the local physicians. The Marlboro milk is bottled last, after the Boston car is filled, and perhaps the infection may have occurred earlier in the run. Again, the Marlboro milk is held for the 20 hours or so between bottling and delivery in a cold-storage room, while the Boston milk is in a freight car, though of course packed in ice. It may be that the Boston milk was not quite so thoroughly cooled and underwent a greater multiplication of the infectious germs. The other point of interest lies in the fact

that while the tonsilitis germ had apparently been spreading in prosodemic fashion in Marlboro and Southboro and Hudson, after it reached Boston in the milk it did not continue to spread in this way, as evidenced by the almost complete absence of secondary cases. The sanitary conditions in the households affected in Boston were not such as to favor prosodemic spread of disease; but this seems hardly competent entirely to account for the phenomena. It is possible that the character of the germ may have been modified by its sojourn in the milk, and modified in the direction of a closer adaptation to a rich food medium. If so, the same changes that made it more virulent for the human beings who ingested it might have made it less able to endure dryness and other unfavorable conditions outside the body, and therefore less likely to be spread in prosodemic fashion. Or, again, the prosodemic spread of tonsilitis may require the action of contributory environmental causes, such as cold or dryness or dust, which ordinarily occur in spring, and such causes may have been lacking in Boston by the end of May. We know that throat diseases do prevail in spring and decrease in summer; and it is significant that the prosodemic tonsilitis which had been prevalent in Hudson, Marlboro, and Southboro for two months ceased at the same time.

CONNECTION BETWEEN THE HUDSON-MARLBORO OUTBREAK AND THE EPIDEMIC IN BOSTON, BROOKLINE, AND CAMBRIDGE.

The general facts, as they have so far been reviewed, appear to indicate that a rather definite form of acute tonsilitis was prevalent in prosodemic form in Hudson, Marlboro, and Southboro all through the months of April and May, while in the second week of May it suddenly appeared as an acute epidemic connected with the distribution of Deerfoot milk in Boston, Brookline, and Cambridge on the one hand, and to a less extent in Marlboro and Southboro on the other.

There are two sources to be considered for an infection of the type under consideration: cattle and human beings. In most of the similar outbreaks of throat disease which have occurred in England it has been found that the cows were suffering from inflammation of the udder and it has been held that the same germ which caused

these conditions produced the throat infection in the human subject. It was natural, therefore, to think first of the possibility of such an origin in the present case. Two reasons, however, appear to militate against such a conclusion. In the first place, Dr. J. W. Robinson, the veterinarian of the Deerfoot Farms Company, made a thorough examination of all the cattle tributary to the Southboro dairy as soon as possible after May 15, without finding a single case of udder disease. In the second place, circumstantial evidence points to an easy possibility of infection from human sources.

The milk of the Southboro dairy, to which statistical evidence points as the carrier of the infection to Boston, is derived from farms lying mostly in the town of Southboro, but some in adjacent sections of Westboro, Framingham, and Marlboro. It is significant that this district was precisely the one place in Massachusetts where tonsillitis is known to have existed in epidemic form during the month of April, 1911. I have records in my canvass of six cases in the Boston region during this month, of five in Westboro, 11 in Southboro, 20 in Hudson, and 68 in Marlboro (Fig. IV). I do not mean of course to imply that no tonsillitis cases occurred in other cities and towns or that only six cases occurred in Boston and Brookline. I do believe, however, that I have canvassed the situation in the adjoining towns—Framingham, Wellesley, Natick, etc.—sufficiently to make sure that there was no unusual excess of tonsillitis there in either April or May. The four cases in Boston and the two in Brookline represent ordinary non-epidemic tonsillitis which happened to be reported by the doctors interviewed, and it is significant that not one of the six is known to have used Deerfoot milk.

The fact that the particular disease in question prevailed among human beings to a notable and unusual degree in precisely the region where the Southboro milk is collected would alone furnish a reasonable presumption that this, rather than a suppositious cattle disease, was the source of infection; and a careful study of conditions in Southboro tends to strengthen this hypothesis.

The examinations made on May 15 by Professor Prescott and Dr. Robinson failed to show a single case of throat disease on any

of the Deerfoot farms. At the Deerfoot dairy building they found, by questioning the men, that half a dozen were suffering from more or less severe throats, but these cases were plainly partakers in the main outbreak and not causal in relation to it. I have found record of other earlier cases, however, three of which are perhaps significant as indicating the presence of infection in the immediate vicinity. One of the first cases on March 31 was the daughter of Mr. B., an engineer at the dairy. He himself came down on April 6, and had a relapse on May 6. Mrs. R., the wife of the man who receives all the milk at the dairy and pours it into the



Fig. IV.
Tonsillitis in Eastern Massachusetts. April.

mixing tanks, had tonsillitis during the first week of May; and at the same time the two children of Mr. H., a farm hand at the Deerfoot Farm itself, were suffering from tonsillitis. None of the persons known to be sick is supposed to have come into contact with the milk, and neither did Mr. H. himself. So far as I am aware, no one who actually handled the milk or the bottles or cans had tonsillitis until May 13, when Mr. X., who washed the cans, fell ill, and his case was more probably a result of the epidemic and not a cause. It is well recognized, however, that, when an infection is spread generally through a community as this throat disease was, among the workers and their families at the dairy and at the farm, there are always "carriers," incipient and walking cases, who, without symptoms of actual disease, are carrying about and dis-

charging virulent germs. Such a carrier case presumably infected the milk, although under the circumstances it is of course impossible to identify the actual link in the chain of infection. It is precisely such dangers as this which have so often rendered vain the most earnest efforts to protect a milk supply; and the chance of overlooking light cases was of course particularly great with a disease like this tonsillitis, which was often mild in character and which had never been known in this country to have been milk-borne.

Wherever and however the infection may have entered, it seems tolerably certain that the germ must have multiplied in the milk

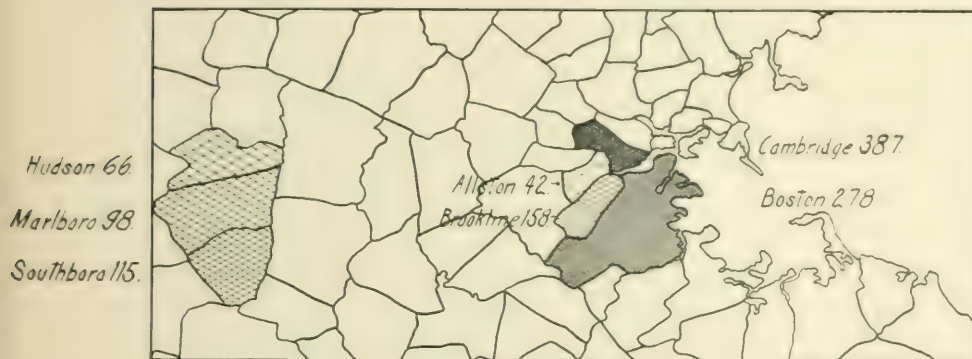


Fig. V.

Tonsillitis in Eastern Massachusetts. May

in order to produce such an extensive and acute infection as that which followed. In Brookline and Cambridge, where my records are most complete, one house in every four supplied with Deerfoot milk is known to have been infected; in 44 per cent of the families there was more than one case, and in 22 per cent, more than two cases.

PRECAUTIONS TAKEN AT THE DEERFOOT DAIRY TO GUARD AGAINST INFECTION.

The milk which caused the epidemic was all morning milk, brought by team to the Southboro dairy a few hours after milking. The cattle at the farms are inspected at least four times a year by Dr. J. W. Robinson, the veterinarian, and the sanitary conditions are regularly inspected by him and are checked by bacterial

analyses, made six to eight times a month, by Professor S. C. Prescott. The stable rules communicated to all farmers call for carding and brushing of cattle at least once daily, and bedding with a clean, dry, absorbent bedding. Stables must be well lighted, well ventilated, and kept clean, and whitewashed at least twice a year. The udders and flanks of the cows are to be thoroughly cleaned before milking, preferably by washing or wiping with a damp cloth. Milking is to be done with dry hands and by milkers in clean clothes, and the milk must be at once removed to a suitable milkroom. Rules in themselves, of course, mean little, but the bacterial results obtained, some of which will be cited later, indicate that these rules have been enforced with considerable success.

The use of milk from any cow that has trouble of any description with her udder is forbidden; and most important of all in connection with the present epidemic, stringent rules are in force against the danger of human disease. Milk from any farm where sickness occurs is not used, but is paid for in full, and every effort has been made to acquaint the farmers with this system. Every year a circular of instructions is sent out containing passages like the following:

In the question of sickness, for instance in cases of typhoid fever, scarlet fever, small pox, or diphtheria, if the farmer announces at the dairy immediately this condition he will be paid the regular price for all his milk, though it is not used. If the information of said sickness should come from outside parties or through the local boards of health first, we shall consider it fair and proper to drop for the time being the milk from any farm where the above conditions exist and shall make no payment for the milk from the time of stoppage until delivery can be resumed.

August 24, 1910, the following letter was sent out to all farmers:

Owing to the prevalence of typhoid fever in the neighboring towns it is necessary that we should take extra precautions to safeguard our milk supply, as milk is a very favorable medium for the growth of this disease. For this reason we think it best to remind you at this time of our promise to pay for all milk withheld in case of sickness from this or any other contagious disease if our patron notifies us himself, and at once, of any such sickness in his household or among his employees. If, however, the information comes from outside sources rather than from our patron, we shall consider it fair and proper to drop his milk for the time being without pay for the same. If there should be symptoms of contagious disease and you are in doubt, if you will call a physician for an examination and send us the bill we will pay the same.

An epidemic of typhoid or other contagious disease traced to our milk would cause a great deal of trouble for all concerned and seriously affect the demand for milk.

Information should not be withheld on account of a light case, as it is quite as likely as a severe case to transmit the disease.

We do not wish our patrons to suffer any financial loss and will see that they do not if they will but follow our suggestions. In case of contagious disease it will merely mean withholding your milk for the time being, sending us a report of the same at the end of the month, and you will receive your cheque the same as if the milk were used. We wish to ask your co-operation with us in this regard as it is the only way that we can maintain our record of never having had an epidemic of any contagious disease traced to our supply.

It is difficult to see what more could have been done along these lines; yet even such precautions failed to prevent the infection of the milk with which we are concerned. It should be noted, of course, that the particular infection was only a sore throat and thus much more liable to escape detection than one of the recognized epidemic diseases; and even so the probability is that the infecting agent was an unrecognized carrier rather than a well defined case.

When the milk arrives at the Deerfoot Dairy it is at once smelled (not tasted) for acidity and then poured into a pipe leading to the mixing tank. This pipe passes through a partition, and the mixing tank itself is in a clean white-painted room which no one enters during the working period. Only the one man who smells the milk and pours it into the pipe comes into contact with it after it reaches the dairy. From the mixing tank the milk passes in closed pipes to a centrifugal clarifier; and thence to a bottling machine, bottling and filling being conducted automatically and without human intervention.

The mixed milk from the Southboro dairy and the separate milk from all the individual dairies are examined by Professor S. C. Prescott, as noted above, and he has kindly furnished me with all his records for the month of May. The results for the mixed Southboro milk are shown in Table 12; the quantitative figures represent counts made after 24 hours at 37°. The samples of milk were also examined after centrifuging for leukocytes and streptococci with no excess of either in any sample.

The counts obtained on the separate samples from the individual farms are of course still lower. Of 1,204 farm samples examined in May (including both Northboro and Southboro supplies) only seven showed over 500,000 bacteria per c.c., 22 between 250,000

and 500,000, and 49 between 100,000 and 250,000, while 604 samples had less than 10,000 and 882 samples had less than 50,000.

I am at a loss to suggest any other precautions that could have been taken to guard against infection with human germs of disease, that were not taken in this instance. Excellent regulations were drawn up for the exclusion of contagion, the farms and cattle were carefully inspected, the dairy was admirably arranged, and the whole process controlled by laboratory examinations under the direction of a bacteriologist and sanitarian of the highest standing. If, in

TABLE 12.
BACTERIA PER C.C. IN SOUTHBORO BOTTLED MILK.

Date		Number in Each of Several Samples		
May	1	200,000	120,000	140,000
	3	23,000	75,000	250,000
	5	30,000	15,000	10,000
	8	35,000		
	10	170,000	200,000	80,000
	15	45,000	70,000	75,000
	18	100,000	150,000	60,000
	24	150,000	150,000	130,000
	26	45,000	300,000	200,000
	29	85,000	95,000	45,000

spite of such precautions, the Deerfoot milk became infected, any raw milk supply may at any time become infected; and this I believe to be the lesson, not only of this outbreak, but of many that have preceded it in all parts of the world. It is practically impossible to exclude mild and unrecognized cases of disease from contact with the process of milk production. The larger a supply, the greater of course is the danger; but even a small supply must meet it at some time. Then a cough over the pail, a finger inside the can as it is lifted, and the danger is imminent.

Boston has suffered severe lessons along this line. In 1907 there were 717 cases of scarlet fever traced to one milk supply and 72 cases of diphtheria to another.¹ In 1908 there was a milk-borne outbreak of typhoid fever totaling 400 cases.² In 1910 there was another scarlet fever epidemic of 842 cases.³ Including the present outbreak there have been over 3,000 cases of epidemic disease traced to milk in the immediate neighborhood of Boston in a period of five years.

There is, in my judgment, but one certain safeguard against such outbreaks—proper pasteurization; but two things must be

¹ *Rep. State Bd. Health, Massachusetts, 1907, 39, p. 489.*

² *Ibid.*, 1908, 40, p. 751.

³ *Monthly Bull. State Bd. Health, Massachusetts, July, 1910, p. 298.*

understood in recommending pasteurization as a general practice. Pasteurization has been too often used in the past by unscrupulous dealers to cover up milk so dirty as to be unsalable without it. Regulations as to sanitary inspection and bacterial counts are just as imperative for milk to be pasteurized as if it were to be sold raw, and the standards should be set just as high as economic conditions permit. In the second place, many processes of pasteurization do not pasteurize. No process should be accepted unless the milk is held at a temperature of at least 145° F. for 20 minutes. The systems of "flash" pasteurization are often worse than useless. Finally, the milk must be properly protected from secondary contamination after pasteurization. The treated milk should either be conducted in closed pipes to an automatic bottling machine; or, best of all, the pasteurization should be conducted in the final package ready for delivery.

The process introduced at the Deerfoot Dairy since the outbreak for the preparation of pasteurized milk for those who desire it, which consists in bottling as usual, capping with a metal cap, submerging in a tank of water and heating for 30 minutes at 150°, seems to me worthy of special commendation, since it excludes all personal contact of attendants, possibly suffering from infectious disease.

SIMILAR OUTBREAKS OF MILK-BORNE SORE THROAT IN OTHER COUNTRIES.

So far as I am aware, no milk epidemic of tonsillitis or similar throat disease has been hitherto reported in this country. In Great Britain, however, the phenomenon has been a common one. Swithinbank and Newman¹ even go so far as to say "it is safe to assume that a year never goes by in which there are not outbreaks of sore throat or tonsillitis due to milk or cream."

Two more or less distinct types of epidemics may be distinguished, those in which the throat disease resembles atypical scarlet fever and is perhaps associated with definite scarlet fever cases, and those, like the one we have been considering, in which tonsillar and peritonsillar infection followed by septic invasion are the chief symptoms. Of the first type was the epidemic at South Kensington in 1875,² where 20 persons who had used cream from a district where 119 cases of sore throat had occurred suffered, some from sore throat and some from scarlet fever. At Oxford in the spring of 1882³ there was a case of scarlet fever in a dairy farm between February 27 and March 3.

¹ H. Swithinbank and G. Newman, *Bacteriology of Milk*, London, 1903.

² *Rep. of Med. Off. to Local Gov't Bd., Great Britain*, 1875, 7, p. 80.

³ *St. Bartholomew's Hosp. Rep.*, 1884, 20, p. 93.

Between March 7 and 15 there developed among 85 persons using this milk 14 cases of sore throat, six of scarlet fever, and one of diphtheria. At Upton and Macclesfield in 1889¹ between January 24 and February 4, 83 cases of sore throat, 38 of scarlet fever, and two of diphtheria broke out among the customers of a single milk supply. Newsholme² reports a small outbreak of nine cases of sore throat and seven of scarlet fever at Brighton, which he believed to be due to milk, on grounds which do not appear altogether conclusive. The interesting thing about these epidemics is the possibility they suggest of a common causative agent for scarlet fever and a comparatively mild throat infection; but they have of course no direct bearing on the epidemic under immediate consideration.

Outbreaks of the second class, definitely of the tonsilitis or quinsy type, are much more common and often present a striking parallelism with the phenomena of the Boston epidemic.

At Aberdeen in 1881,³ 90 families were affected, out of a total of 110 supplied by a single dairy, and in the 90 families there were 300 cases. The onset of the disease was marked by severe rigors followed by fever, and both throat and tonsils were inflamed, but without the formation of a false membrane. After two to four days the fever subsided, leaving great prostration. Relapses were common and the lymphatic glands frequently became swollen and remained enlarged for a long time. Three deaths occurred among old persons.

A similar outbreak was studied by Dr. George Wilson at Rugby School in 1881.⁴ Between March 16 and 18 sore throat broke out in three of eight boarding-houses, about 30 cases to a house. It was found that these three houses alone had a common milk supply and that of 37 houses in the town supplied by the same dealer, 15 were affected. In neither the Aberdeen nor the Rugby outbreak was the source of infection discovered, though the presence of milk from a gargety cow was suspected in the latter case.

In 1884 there was an outbreak in Dover which resembles our own in many respects.⁵ One hundred eighty-eight persons were attacked during a period of four days, and the epidemic occurred in the best districts of the town, and was notably severe among domestic servants. Family incidence was heavy, 31 households having a single case each; 21, two cases; 15, three; seven, four; five, five; three, six; one seven; and one, nine cases. The primary inflammation of the tonsils was often accompanied by a vesicular eruption of the throat and followed by enlargement of the lymphatic glands of the neck. Recovery was much slower than in ordinary quinsy, the cervical glands remaining tender and swollen. Rheumatism, erysipelas, and general septic conditions ensued as complications. Evidence connecting the outbreak with a particular milk supply was clear. In 19 streets every house supplied by the particular milkman was attacked, and in 23 other streets 51 out of 86 houses suffered. The infection of the milk was attributed to the fact that the cows in the dairy in question had suffered from foot-and-mouth disease in January.

G. Sims Woodhead and J. M. Cotterill investigated an outbreak at an educational institution in Edinburgh in 1888,⁶ in which the cows suffered from an epidemic of cow-pox which was supposed to be the source of the human infection. The connection

¹ *Rep. of Med. Off. to Local Gov't Bd., Great Britain*, 1889, 19, p. 89.

² *Jour. Hyg.*, 1902, 2, p. 150.

³ *Brit. Med. Jour.*, 1881, 1, p. 657.

⁴ *Ibid.*, 1881, 2, p. 415.

⁵ *Practitioner*, 1884, 32, p. 467.

⁶ *Brit. Med. Jour.*, 1888, 1, p. 1235.

between cattle disease and human disease appears in most of these instances to be somewhat tenuous, but the circumstantial evidence as to the responsibility of the milk is clear enough, whatever the original source of milk infection may have been. In the Edinburgh outbreak 60 cases of sore-throat developed between October 10 and October 20. The milk from the suspected source was stopped and the epidemic ceased. The use of the milk was resumed on November 7, and in the next five days 25 more cases occurred, and the outbreak was again checked by boiling the milk.

Similar phenomena marked the epidemic at Rothesay in 1890.¹ Eighty cases developed between March 16 and April 2. Intense inflammatory hyperemia of the throat was observed, with patches of exudate in some cases and much enlargement of the glands. Temperature varied from 102° to 105° and great prostration was experienced. Rheumatism and many severe attacks of erysipelas followed as complications and three children died. It was found that two milkmaids at the suspected dairy had had sore throats between March 11 and March 17. On April 2 the use of the milk was stopped, and the epidemic ceased. It was used again on May 6 and by May 10 fresh cases began to develop.

In 1900 J. K. Warry reported an outbreak of septic sore throat in the borough of Hackney.² One hundred fifty-one cases of a septic sore throat, closely resembling that observed in the Boston outbreak, were found in 88 households. In every case there was tonsillitis, but not of the ordinary follicular type; and swelling of the cervical lymphatic glands, more or less unilateral. Temperature was high, prostration great, and convalescence protracted. In one case acute septicemia supervened, followed by a fatal pneumonia, and in two cases acute nephritis set in. The only differences between this outbreak and that of May, 1911, in Boston are that the temperature in the Hackney cases assumed a remittent type with night sweats and that a large proportion of children was affected. One hundred thirty-eight of the 151 cases of sore throat, or 85 per cent, were supplied by a single milk dealer, and a canvass of two selected areas showed 29 per cent and 14 per cent of his houses affected, against 2 per cent and 0 per cent of the houses supplied by other dairymen.

An outbreak at Lincoln in 1902³ showed symptoms somewhat intermediate between typical septic sore throat and the epidemics of atypical scarlet fever. Seventy-five cases developed in a single week in the month of May, all but one having a common milk supply. Besides the sore throat and swelling of the cervical glands there was noted erythema of the face, edema of the fauces and uvula, and a coating of drab-colored fur on the tonsils. About two-thirds of the cases exhibited a roseolous papular eruption, and desquamation occurred in a third of the cases. Joint infections and peritonitis followed as complications.

An epidemic of 42 cases of septic sore throat in 22 families at Bedford⁴ showed symptoms more like those of influenza, severe pains and some gastric disturbance, with a temperature of 102° to 103°, accompanying the inflammation of throat, fauces, palate, and uvula. Every case used milk or cream from the same dairy. Twice as many females as males were affected, and 22 of the 42 cases were under 20 years of age.

It may be noted that in none of the last three outbreaks, at Hackney, Lincoln, and Bedford, was any original source of infection either human or bovine demonstrated at the farm or dairy from which the infected milk was derived.

¹ *Glasgow Med. Jour.*, 1890, 34, p. 241.

² *Ann. Rep. Med. Off. of Health, Hackney*, 1900.

³ *Lancet*, 1902, 2, p. 1391.

⁴ *Ann. Rep. Med. Off.*, Bedfordshire County Council, 1902.

An outbreak of septic sore throat at Woking in 1903¹ was of the same general type as the Boston epidemic. Two hundred fifty cases and eight deaths occurred in the months of October and November. Many of the throats were of the ordinary follicular type, others showed a definite membrane suggesting diphtheria, others still would have been classed as quinsy. The temperature was high and accompanied by grippy pains and the cervical glands were frequently involved and were slow to heal. Rheumatic affections of the joints, erysipelas, and peritonitis occurred as complications. Adults were chiefly affected and the course of the disease was more severe in them. It was found that the cases were concentrated on the routes of two dealers, both of whom derived a portion of their milk from a single farm. On this farm four cows were found yielding from certain teats immense quantities of pus and streptococci. The farmer had suffered from quinsy in the middle of September, followed by joint pains, and his wife and four children had been attacked in October.

In an epidemic of sore throat among the staff of the Belvidere Hospital, at Glasgow, there was evidence of bovine infection.² A new cow was added to the herd on April 23. The cows associated with the newcomer quickly developed a teat eruption until by May 6, 30 per cent of the herd had been attacked. Thirty-nine cases of sore throat appeared among the users of the milk during the month of May and the hands of four milkers were found to be affected with sores.

In the same year an outbreak of 100 cases was reported by Robb³ at Paisley but with little detail. There was acute inflammation of the throat with enlarged tonsils and a diphtheria-like membrane, pains, severe toxemia, and great prostration. The "only common factor known was milk supplies" and the cattle had recently suffered from cowpox.

An epidemic at Colchester in 1905⁴ again resembled the Boston outbreak. One hundred forty cases occurred during the month of April in one of the best residential sections of the town. Many servants were attacked, and, among those whose sex was recorded, were 43 adult females, against 13 adult males and 18 children. The incubation period was about two days. Onset of the disease was rapid, with high temperature, often 104° or 105°, and pains in the limbs. The throat was red and swollen, with follicular plugs which sometimes ran together to form a diphtheria-like membrane. The submaxillary glands were enlarged and painful, sometimes leading to a condition of quinsy. There were few secondary cases. In the neighborhood affected, 97 per cent of the cases had used the suspected milk and a house-to-house canvass showed that, of households supplied with this milk alone, 51 per cent were attacked, of those supplied with this and other milk, 33 per cent were attacked, and of those supplied only with other milk, 6 per cent were attacked. There occurred also, simultaneously, 60 cases in the army barracks supplied with the same milk. On one of the tributary farms a cow was found with a diseased udder yielding pus, and on this farm six cases of the disease occurred during the course of the epidemic.

No attempt has been made to search the literature systematically and references to the papers describing most of the outbreaks cited were originally obtained from Swithinbank and Newman's *Bacteriology of Milk* or from *Bulletin 56* of the U.S. Public Health and Marine Hospital Service. Excluding the outbreaks complicated by scarlet fever, 12 epidemics of sore throat disease have been briefly reviewed. In certain cases the symptoms were somewhat different from those observed in the Boston-

¹ *Jour. State Med.*, 1904, 12, p. 595.

³ *Ibid.*, 1904, 17, p. 773.

² *Pub. Health*, 1904, 16, p. 769.

⁴ *Ibid.*, 1905, 18, p. 1.

Brookline-Cambridge outbreak. In other cases, as at Aberdeen, Dover, Rothesay, Hackney, Woking, and Colchester, both primary symptoms and secondary complications were so similar as to make it appear highly probable that we are dealing with the same quite definite disease. In four of the 12 outbreaks there was no evidence whatever as to the original source of infection, and in four other cases there was a dubious connection, only, with some bovine disease. At Glasgow and Colchester there was fairly strong circumstantial evidence of a connection with inflammation of the cow's udder. At Rothesay probability pointed to human infection, and at Woking there had been both human quinsy and bovine udder inflammation on the farm.

SUMMARY AND CONCLUSIONS.

A sudden outbreak of a peculiar form of acute tonsillitis, or septic sore throat, occurred in Boston and its vicinity during the month of May, 1911. Suspicion was directed toward a certain milk supply, that of the Deerfoot Farms, but there were puzzling circumstances which led to a difference of opinion and to a suspension of judgment in official circles. At the request of the officers of the dairy company, I made an investigation of the statistical and epidemiological side of the problem during the summer months.

The disease was not ordinary follicular tonsillitis, but more nearly what the English recognize as septic sore throat. In early stages there was merely a diffuse redness over the tonsils and adjoining regions, but follicular patches often appeared later and in many cases a membrane simulating that of diphtheria. Peritonsillar abscesses and enlarged cervical glands, of a stubborn nature, marked the second stage of the disease, and these were followed by diverse complications—rheumatism, erysipelas, nephritis, pericarditis, pneumonia, pleurisy, peritonitis, and general septic conditions. The disease was severe, and occasionally fatal among the old and weak. Inquiries made by the district inspectors of the state board of health and supplemented by my own investigations made it clear that the disease in question, so far as any abnormal epidemic prevalence was concerned, was confined to two definite foci centering respectively about Boston on the seacoast and about Marlboro, 25 miles to the westward.

The Boston epidemic affected the Back Bay and other regions of Boston, the town of Brookline, and the city of Cambridge. Through the courtesy of the Boston and Brookline boards of health and by personal interviews with physicians (over 80), I have

obtained records of 1,043 cases in this vicinity. They form a sharply marked epidemic, beginning on May 8, rising to a maximum on May 14, and practically ceasing after May 22. There were few secondary cases and a single common source of infection for the three communities is clearly indicated. Cases were concentrated in the families affected, only 56 per cent of the households having a single case and 22 per cent having three cases or more. Females suffered twice as much as males. Adults suffered more in proportion than children. Only 15 per cent of the cases were under 16, 44 per cent between 16 and 35, 23 per cent between 36 and 55, and 17 per cent over 55. Forty-eight deaths were attributed to the epidemic, but in some cases the fatal complications were somewhat remote. Two-thirds of the fatalities occurred at ages above 55, and one-third at ages above 75.

It soon appeared that the distribution of the epidemic exactly coincided with that of one of two main milk supplies of the Deerfoot Company. It affected the particular districts in Boston, Brookline, and Cambridge where this milk was used, and it broke out simultaneously in Marlboro and Southboro, the only other towns to which it was distributed. The cases obtained from physicians in Boston and Cambridge were compared with the delivery lists of the dairy, and over 85 per cent were found there, while an additional 8 per cent were stated by physicians to have used the milk, though not listed as subscribers. The Deerfoot supply makes up about 1 per cent of the Boston and 2 per cent of the Cambridge total. In Brookline my records include all cases of tonsillitis reported in answer to a circular sent out to all physicians and covering a period of five weeks. These data, including not merely the epidemic, but ordinary tonsillitis as well, showed 65 per cent of the cases on the Deerfoot lists and 8 per cent more stated to have used the milk. The Deerfoot supply was about 7 per cent of the total for the town. A study of the delivery lists for Brookline and Cambridge, where my records were fairly complete, showed that in each case about one family out of every four supplied had been infected.

The other outbreak, in the region of Marlboro, seemed at first to complicate the situation. The disease which prevailed there was exactly the same type which occurred in Boston and I obtained

records of 392 cases in the three towns of Hudson, Marlboro, and Southboro. The household incidence was much less marked than in Boston, and instead of a large excess among females there was a slight excess among males. A study of the dates of onset indicated that the epidemiological conditions were entirely different from those in Boston. Instead of a sudden explosive outbreak the disease was present all through the months of April and May, and was evidently spreading from person to person in ordinary prosodemic fashion without any single common bond. In Hudson, where Deerfoot milk is not sold, there was a fairly even distribution over a period of five weeks. In Marlboro and Southboro, on the other hand, in addition to the general prevalence of the whole period, there was a special outbreak simultaneous with that in Boston, during the second and third weeks of May. While the cases occurring during the other six weeks in Marlboro were chiefly among the users of other milk supplies than Deerfoot, the excess in the epidemic period was wholly among Deerfoot customers. In Southboro there was at this time an epidemic of 64 cases in two boys' boarding-schools supplied with Deerfoot milk.

It appears, then, that the peculiar type of tonsillitis in question prevailed in prosodemic form in Hudson, Marlboro, and Southboro during April and May, and in the second week in May appeared as a sharp epidemic, following the Deerfoot milk, in Boston, Brookline, and Cambridge, and in Marlboro and Southboro. The Deerfoot milk associated with the epidemic is derived from farms in Southboro, Marlboro, and the adjacent regions, and probability points to an infection of the milk from the human cases known to be so abundant in this neighborhood. No record has been obtained of any well defined case of tonsillitis in direct contact with the milk. Cases are known to have occurred, however, at the proper time in a family on the Deerfoot Farm and in the families of employees at the Deerfoot Dairy. It is probable, therefore, that the actual infection was due to a carrier case. No cattle disease is known to have occurred on any of the farms.

I am unable to find that any of the natural precautions which could be taken to prevent infection of the Deerfoot milk had been neglected. Farms and cattle were systematically inspected by an

expert veterinarian. It was well understood that milk from a farm where contagious disease existed should be paid for and not used. Facilities for handling the milk at the dairy were of the best, and the whole process was controlled through frequent bacteriological analyses by a sanitary expert of the highest standing. The lesson to be drawn from the outbreak is that even a most carefully supervised milk supply is open to the danger of grave infection from carrier or unrecognized cases of disease. The only real safeguard against such catastrophes lies in pasteurization, carried out by the holding system and preferably in the final packages.

Numerous outbreaks of similar throat disease have occurred in Great Britain, and have been clearly traced to infected milk supplies. From the English experience it appears that "septic sore throat" is by no means rare as a milk-borne infection; and sanitarians in this country must add this to the list of dangers that surround a raw milk supply.

ON THE PRODUCTION OF ANAPHYLATOXIC SUBSTANCES BY AUTOLYSIS OF BACTERIA AND THEIR RELATIONS TO ENDOTOXINS.*

E. C. ROSENOW.

(From the Memorial Institute for Infectious Diseases, Chicago.)

In a recent article¹ I have shown that when virulent pneumococci are suspended in NaCl solution it becomes very toxic for normal guinea-pigs at a certain period of autolysis, and intravenous injections produce the symptoms and changes characteristic of anaphylactic shock. These results speak strongly in favor of the view that the symptoms in sensitized animals following a second injection of pneumococcus extract are due to a rapid splitting of the protein into toxic material and that a similar splitting occurs at a much slower rate *in vitro* by autolysis.

In the following pages I wish to report briefly the results of similar experiments made with the streptococcus pyogenes and mucosus, the staphylococcus, the typhoid bacillus, the colon bacillus, the bacillus pyocyaneus, the meningococcus, the gonococcus, the dysentery bacillus (Shiga), and the spirillum of Metchnikoff. The bacteria were grown on the surface of ascites agar (the ascites fluid used was heated first at 60° C. for 24 hours), Loeffler's blood serum slants, or in meat broth. They were suspended in NaCl solution so that each cubic centimeter represented approximately 2.5 billion bacteria. When the bacteria were grown in broth they were first washed in NaCl solution.

The two strains of streptococci were recently isolated, one from the blood in a case of puerperal sepsis, the other from the throat of a case of scarlet fever. The streptococcus mucosus came from the sputum in a case of bronchial asthma and was only slightly virulent. It underwent autolysis very slowly in NaCl solution, formed long chains, and had lost its capsule. The meningococcus was isolated in pure culture from the cerebrospinal fluid of a case of meningitis six weeks previously. For the strains of gonococcus I am indebted to Dr. Irons. One strain was isolated a long time ago, the other was in the seventh generation. The strain of colon bacillus, and *B. pyocyaneus* were isolated from cases of cystitis, while the typhoid bacillus and the spirillum of Metchnikoff were taken from laboratory stock cultures. The strain of Shiga bacillus was isolated from a case of dysentery by Dr. Dick.

* Received for publication November 23, 1911.

¹ *Jour. Infect. Dis.*, 1911, 9, p. 190.

All injections, the total quantity being always 7 c.c. unless otherwise indicated, were made into the jugular vein of normal guinea-pigs weighing from 200 to 250 gms. The ether, when previously added, was always removed before injection. Control experiments showed that it makes no difference so far as the immediate symptoms are concerned whether the bacteria are removed or not; hence centrifugation was omitted in most instances. In this way it was possible also to study the later effects of the bacteria injected. The symptoms produced and the postmortem appearances are identical with those of anaphylaxis and those following the injections of substances obtained from pneumococci, whether by autolysis in NaCl solution or by treating them with immune and normal serum. The typical convulsions, respiratory spasm, sudden drop in temperature, leukopenia followed by fever if the animal survives the acute symptoms are indistinguishable from anaphylaxis. Extreme emphysema of the lungs, ecchymosis, occasional hemorrhages into the lungs and heart, especially if the animal survives a number of hours, and dark blood due to asphyxia are found postmortem.

EXPERIMENTS WITH STREPTOCOCCUS PYOGENES.

Numerous experiments with extracts of the two strains of virulent streptococci indicate that it is difficult to obtain highly toxic substances from streptococci in NaCl solution. Extracts from suspensions kept at 37° and 15° C., with and without ether, were tested at intervals of from 24 hours to 11 days. All proved non-toxic except two in which extraction had been carried out under ether at 37° C. for six days and 10 days respectively. The former produced definite although not fatal symptoms in two normal guinea-pigs when 3.5 c.c. were injected, while the latter produced typical symptoms with death in two minutes when 7 c.c. were injected into each of two guinea-pigs. In Table 1 it is shown further that while it is difficult to obtain an active poison from streptococci even by means of immune¹ and normal serum it is nevertheless possible, the results of Paul Th. Mueller² to the contrary notwithstanding.

¹ Parke, Davis & Co.

² *Ztschr. f. Immunitätsf.*, 1911, 10, p. 164; *ibid.*, 1911, 11, p. 200.

Streptococci from fresh broth cultures yield little or no toxic substance when treated the first time in immune serum and then in normal serum, but when previously treated with sodium oleate 1:1,000 or when the same number of streptococci from old broth cultures are used, they yield toxic substances. If fresh streptococci, on the other hand, are treated in normal guinea-pig serum

TABLE 1.
EXPERIMENTS WITH STREPTOCOCCUS PYOGENES.

Mixtures Injected	Symptoms in Normal Guinea-Pigs	Appearance of Cocci at the Time of Injection
Strept. from 30 c.c. broth + 0.5 c.c. immune serum at 37° C. for ½ hour + 4 c.c. normal guinea-pig serum at 15° C. 48 hours	No symptoms	Nearly all gram positive
Strept. from 30 c.c. broth + 0.5 c.c. immune serum at 37° C. for 1½ hours + 4 c.c. normal guinea-pig serum at 15° C. 48 hours	Slight symptoms. Drop of 2.2° F.	Nearly all gram positive
Strept. from 30 c.c. broth + ether 37° C. 1 hour + 0.5 c.c. immune serum at 37° C. for ½ hour + 4 c.c. normal guinea-pig serum at 15° C. 48 hours	No symptoms	Many gram negative
Strept. from 30 c.c. broth + 1/1,000 sodium oleate 37° C. 1 hour + 0.5 c.c. immune serum at 37° C. for ½ hour + 4 c.c. normal guinea-pig serum at 15° C. 48 hours	Slight symptoms. Drop of 3.6° F.	Many gram negative
Strept. from 30 c.c. broth + 0.5 c.c. immune serum at 37° C. for 6 hours + 4 c.c. normal guinea-pig serum at 15° C. 48 hours	Slight symptoms	More than one-half gram negative
Strept. 21 days old from 30 c.c. broth + 0.5 c.c. immune serum at 37° C. for ½ hour + 4 c.c. normal guinea-pig serum at 15° C. 48 hours	Severe symptoms	More than one-half gram negative
Strept. from first five mixtures suspended in 20 c.c. fresh guinea-pig serum at 37° C. 3 c.c. injected at the end of 1½ hours	Severe symptoms. Nearly dead in 3 minutes	More than one-half gram negative
Strept. from first five mixtures suspended in 20 c.c. fresh guinea-pig serum at 37° C. 3 c.c. injected at the end of 3½ hours	Death in 2 minutes	More than one-half gram negative
Strept. from first five mixtures suspended in 20 c.c. fresh guinea-pig serum at 37° C. 3 c.c. injected at the end of 8 hours	No symptoms	More than one-half gram negative
Strept. from first five mixtures suspended in 20 c.c. fresh guinea-pig serum at 37° C. 3 c.c. injected at the end of 8 hours + 1.5 c.c. fresh guinea-pig serum	Death in 3 minutes	More than one-half gram negative
Control-guinea-pig serum 37° C. 8 hours	No symptoms	
Control-guinea-pig serum 15° C. 48 hours	No symptoms	

The strain of streptococcus used in these experiments was obtained from the blood in a case of puerperal sepsis and was moderately virulent to guinea-pigs. The cocci were grown in meat broth for 48 hours. Rosenow's capsule stain was used to bring out the change in the streptococci. Subcultures on blood agar proved that no contamination had taken place in the mixtures at the time of injection.

a second time after one treatment in immune serum, highly toxic substances are obtained. The table shows that in one and one-half and three and one-fourth hours at 37° C. the toxicity is marked, while at the end of eight hours the toxicity has disappeared. If fresh guinea-pig serum is added to this serum extract which has become harmless the mixture at once becomes toxic again. The toxic

substance appears in solution when the streptococci show definite tinctorial and morphological changes just as is the case with pneumococci.

Extracts of streptococci in NaCl solution which have not yet had time to become toxic have been found to yield toxic substances promptly by the addition of one part of normal serum to three parts of salt solution. The changes which take place in streptococci allowed to age in cultures and treated with sodium oleate, or suspended in NaCl solution, are probably all of the same character as when treated in immune serum, because one treatment in serum in these instances is sufficient to bring into solution the toxic substances, whereas with fresh streptococci a second and longer treatment is necessary. From these results it would seem that streptococci in the serum mixture furnish at least a part of the anaphylatoxic substance just as they are its only source in the NaCl solution. In both instances the time required, in order to bring the toxic substances in solution, is greater than in the case of virulent pneumococci.

EXPERIMENTS WITH STREPTOCOCCUS MUCOSUS.

Table 2 shows that the streptococcus mucosus also yields a toxic substance or substances when extracted in NaCl solution under

TABLE 2.
EXPERIMENTS WITH STREPTOCOCCUS MUCOSUS.

Seven c.c. of the NaCl Solution Extract after Treatment as Mentioned Injected Intravenously in Normal Guinea-Pigs Weighing 250 Grams	Symptoms
Str. mucosus in NaCl sol. at 37° C. for 1, 2, 4, 9, and 28 days.....	No symptoms
Str. mucosus in NaCl sol. at 37° C. for 9 days, 5 c.c. + 2 c.c. normal guinea-pig serum at 37° C. for 1½ hours.....	Severe symptoms
Str. mucosus in NaCl sol. + ether at 37° C. for 1, 2, and 4 days.....	No symptoms
Str. mucosus in NaCl sol. + ether at 37° C. for 9 days.....	Severe symptoms
Str. mucosus in NaCl sol. + ether at 37° C. for 9 days, 5 c.c. + 2 c.c. normal guinea-pig serum at 37° C. for 1½ hours.....	Death in three minutes
Boiled str. mucosus in NaCl sol. + ether at 37° C. for 4 days + washed guinea-pig leukocytes in ice-chest over night and at 37° C. for 5 hours.....	Death in four minutes
Boiled str. mucosus in NaCl sol. + ether at 37° C. for 4 days + washed guinea-pig leukocytes in ice-chest over night and at 37° C. for 13 hours.....	No symptoms

The leukocytes were obtained from guinea-pigs injected 24 hours previously with aleuronat. The NaCl solution suspension of leukocytes gave no symptoms at the end of 24 hours at 37° C. but did at the end of 48 hours. Cultures proved that the mixtures remained sterile.

ether. When the organisms are suspended in NaCl solution at 37° C. changes take place before the toxic stage is reached which are probably similar in nature to those produced by immune serum,

because normal serum renders the NaCl suspensions which have been kept at 37° C. nine days highly toxic in one and one-half hours at 37° C. just as with cocci previously treated in immune serum. The addition of washed guinea-pig leukocytes to the partially autolysed extracts bring into solution promptly the toxic substance and at the same time converts it into harmless products in eight hours after the height of toxicity has been reached.

The toxicity of the leukocytic suspension in NaCl solution which develops at the end of 48 hours is interesting. It seems as if during disintegration the leukocytes yield toxic substances, although the possibility that this is formed from the small quantity of aleuronat remaining in the exudate, must be borne in mind.

EXPERIMENTS WITH TYPHOID AND COLON BACILLI.

Table 3 shows that the typhoid bacillus yields a toxic substance when suspended in NaCl solution under ether at 37° C. Here

TABLE 3.
EXPERIMENTS WITH TYPHOID BACILLI.

Seven c.c. of the NaCl Solution Extract after Having Been Treated as Indicated Are Injected	Symptoms in Normal Guinea-Pigs	Appearance of Bacilli by Giemsa Stain	Number of Viable Bacilli per Loop
Bacilli in NaCl sol. at 37° C. for 2 hours.	No symptoms. No change in temperature	One-half stain deeply, the rest faintly	Innumerable
Bacilli in NaCl sol. at 37° C. + ether for 2 hours.	Death in 6 minutes from typical shock. Drop of 6° F.	All stain faintly	14
Bacilli in NaCl sol. at 37° C. + ether for 48 hours.	Slight symptoms. Drop of 1.5° F.	All stain faintly	6
Bacilli in NaCl sol. at 37° C. + ether for 72 hours.	No symptoms. Rise of 0.6° F.	All stain faintly	6
Bacilli suspended in NaCl + washed guinea-pig leukocytes at 37° C. for 22 hours.	Severe symptoms. Drop of 2° F.	Mostly faintly staining	Undetermined
Bacilli suspended in NaCl + washed guinea-pig leukocytes at 37° C. for 48 hours.	No symptoms	Mostly faintly staining	Undetermined

Washed leukocytes give no symptoms in 22 hours but death in three minutes when kept at 37° C. for 48 hours.

there is also a close relation between disintegration and death of the bacilli and the appearance of the anaphylatoxic substance in solution. As with the streptococcus mucosus, leukocytes rapidly bring into solution the toxic substance and render it harmless again.

Similar experiments with colon bacillus gave approximately the same results. However, instead of the toxicity in NaCl solution under ether developing in 22 hours it appeared first at the end of 48 hours. The tinctorial changes and death of the bacilli appear at a correspondingly later period.

EXPERIMENTS WITH STAPHYLOCOCCUS.

Experiments were made with extracts in NaCl solution of staphylococcus aureus but with negative results. Suspensions both with and without ether were tested at intervals of from five hours to 26 days. Stained specimens show that the cocci are well preserved and mostly gram positive even at the end of this long period. Mixtures of washed guinea-pig leukocytes and staphylococci in NaCl under ether at 37° C. for 48 hours, however, proved fatal with typical symptoms in three minutes. The corresponding control suspensions of leukocytes in NaCl solution both with and without ether became only moderately toxic after 48 hours at 37° C. and then at 15° C. for five additional days. Staphylococci from 20 c.c. broth treated in 2 c.c. antistreptococcus serum at 37° C. for one hour and then suspended in 4 c.c. normal guinea-pig serum in ice-chest for 20 hours, and then at 37° C. for eight hours, caused death with typical symptoms in four minutes. A similar toxic substance was obtained when 3 c.c. of fresh guinea-pig serum was added to 4 c.c. of extract of staphylococcus in NaCl solution which had been kept at 37° C. for four days, this mixture being kept at 37° C. for 20 hours previous to injection. The control mixture of NaCl solution and serum remained permanently harmless.

In other words, while it has not been possible to obtain any anaphylatoxic substance from staphylococci in NaCl solution it is easily brought in solution by means of leukocytes and serum.

EXPERIMENTS WITH MENINGOCOCCUS AND GONOCOCCUS.

The experiments with the meningococcus were made by testing the toxicity of suspensions in NaCl solution with and without ether after being kept at 37° C. 10 minutes, one hour, 20, and 48 hours. The extracts which were prepared without ether were toxic at an earlier period than those to which ether was added.

The toxicity also disappeared earlier. Thus definite symptoms followed the injections made after 10 minutes and fatal symptoms those made after one hour, but there were no symptoms from the injections made at the end of 20 hours when no ether was added; when ether was added the earlier extracts were less toxic but produced unmistakable symptoms after remaining at 37° C. for 48 hours. The animals receiving the suspensions before the toxic substance had gone into solution died at the end of 48 hours with no gross lesions; those which received the suspensions but survived the severe immediate symptoms died in 18 hours with ulcer of stomach and marked postmortem digestion of the gastric mucous membrane, while those receiving the suspensions after the toxicity had disappeared remained permanently well.

Experiments with extracts in NaCl solution of the two strains of gonococci gave similar results. The more recently isolated strain disintegrated more rapidly in NaCl solution, was more difficult to grow, and yielded a highly toxic substance in 15 minutes after the suspensions in NaCl solution were made. The toxicity was greater and disappeared at a correspondingly earlier period than that of the strain which had been cultivated for a much longer period. The former killed the guinea-pigs in the usual way, the latter produced severe symptoms. The injection which produced definite and immediate but not fatal symptoms killed within 24 hours with ulcer of stomach as the chief postmortem finding, while the animals receiving the suspensions after the toxicity had disappeared survived.

EXPERIMENTS WITH THE SPIRILLUM OF METCHNIKOFF.

Extracts in NaCl solution of this spirillum, previously grown in plain broth for 20 hours, produced marked symptoms in 10 minutes after the suspensions were made, but no symptoms were obtained at the end of four and 24 hours. The early appearance of the toxic substance in NaCl solution with this microorganism is in keeping with the small amount of culture necessary to obtain positive results when treated with serum. Two loops from the surface growth on agar treated in antistreptococcus serum at 37° C. for one-half hour and then digested in 4 c.c. normal guinea-pig serum on ice 48 hours,

as well as the same amount of culture suspended directly in normal serum, gave the toxic action. The toxicity of both these mixtures disappeared completely after exposure to 37° C. for three and one-half hours.

EXPERIMENTS WITH THE BACILLUS PYOCYANEUS AND THE DYSENTERY BACILLUS.

Repeated attempts were made to obtain toxic substances from the bacillus pyocyaneus but with negative results. Although immediate symptoms suggestive of anaphylactic shock could not be obtained, the suspension caused death in 18 to 48 hours. This toxicity remained for six days after the suspensions were made, differing in this respect from the effect of NaCl solution suspensions of the bacteria producing no soluble toxin in that the latter no longer intoxicate when they have lost the power of causing immediate symptoms.

NaCl solution extracts of the Shiga bacillus of dysentery gave results exactly similar to the bacillus pyocyaneus.

It is well known that the difference in the amounts of bacteria necessary in order to obtain the anaphylatoxic substance in a given time when treated with immune serum and complement is very great. Several loops of typhoid bacilli or cholera vibrio and of dysentery bacilli all susceptible to serum lysis will produce as much intoxication as pneumococci, streptococci, or staphylococci (bacteria which are not susceptible to serum lysis) from 30 c.c. or more of a broth culture. These observations show that bacteria which disintegrate rapidly in NaCl solution also yield the toxic substance at a correspondingly earlier period and from smaller amounts than those which disintegrate slowly. This is true of various strains of the same species as well as of the different species. In case of the pneumococcus there is a close relation between virulence, the rate of disintegration, and the time of appearance of the anaphylatoxic substance in NaCl solution and when treated with immune and normal serum. Streptococci resist disintegration in serum and NaCl solution much longer than virulent pneumococci and yield the toxic substance at a correspondingly later period. Heat-

killed bacteria such as meningococci, gonococci, and typhoid bacilli, and others susceptible to serum lysis are toxic to animals to a much higher degree, and as shown here yield toxic substances more quickly than pneumococci, streptococci, and staphylococci.

The rapidity with which bacteria disintegrate in NaCl solution or in cultures is generally believed to be due to the activity of an autolytic ferment, the so-called "endotryptase."

As pointed out, the quicker disintegration occurs the sooner the toxic substance appears in solution, hence it probably is a split product of the bacterial protein. It is interesting to point out also that there is a certain parallelism between the ease with which the various cocci disintegrate in NaCl solution, with liberation of the toxic substance, and the tenacity with which they retain the stain when treated by the Gram method. Gonococci and meningococci are gram negative. They disintegrate and yield the toxic substance very rapidly. Pneumococci, streptococci, and staphylococci, while gram positive, retain the stain, and resist disintegration in NaCl solution in the order named. Virulent pneumococci when suspended in NaCl solution are largely gram negative in 24 hours, streptococci in a week or more, while staphylococci remain gram positive for two weeks or a month.

As further indication that the toxic substance is really a split product and that microscopic evidence of disintegration of the bacteria is not simply an expression of direct solubility of the bacterial cell and not of proteolysis we have the following results: NaCl solution extracts of the various bacteria, given in Table 4, were prepared by grinding them in the cold and filtering, and the protein content was determined at once and later by formalin titration and by finding the optical activity with the polariscope. As shown in Table 4 the evidence of protein splitting, indicated by the formalin method and by a diminished rotatory power as the various extracts are kept at 37° C., is roughly proportionate to the time required to bring the toxic substance in solution. It is most rapid in case of the typhoid bacillus, less with pneumococcus extracts, and slowest with streptococcus, while in case of the staphylococcus and the pyocyaneus bacillus, from which the anaphylatoxic substances could not be obtained, practically no hydrolysis took place.

I am aware that the accuracy of the formalin titration method for the amount of splitting of protein has been questioned and that changed optical activity of solutions of proteins is not necessarily an accurate index of the degree of proteolysis. The objections raised probably do not apply here, because in the case of pneumococcus extracts control experiments prove that when formalin titration shows definite increase in polypeptids, etc., Nessler's test shows an increased quantity of ammonia in the extract which has undergone autolysis. The coagulable nitrogen (phosphotungstic acid) is also perceptibly diminished.

TABLE 4.
PROTEOLYSIS IN NaCl SOLUTION EXTRACTS OF VARIOUS BACTERIA AS DETERMINED BY FORMALIN
TITRATION AND THE POLARISCOPE.

NaCl SOLUTION EXTRACT OF	FORMALIN TITRATION (Figures represent the part of a c.c. of $\frac{n}{10}$ NaOH required to restore pink of the neutral- ized mixture of extract and formalin)					LEVO-ROTATION IN DEGREES			
	At Once	24 Hours	48 Hours	72 Hours	6 Days	At Once	48 Hours	72 Hours	6 Days
Virulent pneumococci.....	0.5	0.6	0.8	0.8	0.05	0.03	0.025
B. typhosus.....	0.3	0.4	0.10	0.02
St. pyogenes aureus.....	0.25	0.2	0.06	0.05
Str. pyogenes.....	0.5	0.5	0.6	0.07	0.10	0.065
B. pyocyaneus.....	0.3	0.35	0.5	0.03	0.025	0.037

These facts and the results indicated in Table 4 show that the toxic substance is probably a split product of the bacterial proteins. It might be possible that other bacterial substances play a rôle, but that it is not merely a question of getting into solution enough substance to give the symptoms and that it must concern definite chemical changes is further indicated by the fact that the toxicity disappears on longer exposure to 37° C. That the lipoids in some way play a rôle is indicated by the fact that the addition of ether promotes the bringing into solution of the toxic substance. It is likely, however, that ether only serves to kill the bacteria and thus intensifies autolytic changes. This explanation is in harmony with the results of Friedberger and others who find that it is easier to obtain the anaphylatoxic substance from heat-killed bacteria than from living ones by means of immune and normal serum.

The following facts indicate that bacteria are converted into toxic material during infections just as they are by autolysis in NaCl solution. Immune and normal serum and leukocytes bring into solution promptly the toxic substances; formalin titration indicates that the changes are all of a proteolytic nature. Not only are similar toxic substances formed during experimental infections but, as shown by Friedberger, the time of their appearance and disappearance is earlier in immunized than normal animals, and in the case of pneumococcus infections I have repeatedly obtained the toxic substance from the peritoneal exudate of animals, from the blood of animals dying with pneumococcemia, in human beings from pneumonic lungs immediately after death, and also from pus in one case each of pneumococcus empyema and arthritis. The fact that the symptoms of natural infections are different from those in the guinea-pig following the injection of highly toxic extracts is no indication that similar toxic substances may not be formed during infections, because the amount of the toxic substance in solution may not be great enough at any one time to produce the severe reaction. Injection into man of dead bacteria which are subject to serum lysis, such as meningococci, may be followed by extremely severe symptoms, as shown by Davis.¹

As an indication that the toxicity for the guinea-pig may be considered a measure of the toxicity for man it should be pointed out as I have previously shown,² that pneumococci from which the toxic substance has been removed by autolysis in NaCl solution lose practically all their toxic action for man and the guinea-pig. The extract now contains the toxic substance, intravenous injection into normal guinea-pigs giving symptoms indistinguishable from anaphylaxis and subcutaneous injection into man, some fever, marked local reaction, and leukocytosis. Heating this extract to 60° C. for one hour destroys the toxic action for both species. If the autolysis is allowed to continue the toxic action again disappears simultaneously for guinea-pig and for man.

The results of these experiments would seem to furnish the necessary proof that bacteria themselves furnish toxic material when

¹ *Jour. Infect. Dis.*, 1907, 4, p. 558.

Jour. Am. M. Ass., 1910, 54, p. 1943; *Jour. Infect. Dis.*, 1911, 9, p. 190.

treated with immune and normal serum, contrary to the views of Wassermann and Keysser,¹ and that complement, while an active agent, is not absolutely essential for the production of anaphylatoxin, as Friedberger maintains and as the work of Moreschi and Vallardi² and others suggest. The view of Friedberger that the production of anaphylatoxin is not dependent on bacteriolysis, as urged also by Neufeld and Dold,³ does not seem warranted, because the appearance of toxic substances in solution in case of each of the species studied is closely associated with definite evidence of disintegration of the bacteria. I find, it is true, that complete lysis of pneumococci in weak solutions of bile salts (0.5 per cent) and of typhoid bacilli and cholera germs in serum, as Neufeld and Dold have shown, interferes with the production of anaphylatoxic substance, hence it may be that while complete bacteriolysis destroys substance, partial lysis is necessary to produce the toxic substance. The possibility that other solvents such as the lipoids may serve to extract from bacteria the toxic substance more rapidly or at an earlier period during their disintegration is indicated by the results of Neufeld and Dold, who claim to have extracted it by means of lecithin from living typhoid bacilli and cholera vibrio. That death of some organisms must have occurred in their experiments is more than likely, and the function of the lecithin here may have been that of a solvent, making penetration through the cell membrane easier.

From my results it is clear, too, why intravascular injections of dead bacteria or bacterial extracts are much more toxic than subcutaneous and even intraperitoneal injections; in the former case the toxic products are carried rapidly to the cells affected as soon as formed, whereas on the latter modes of introduction the bacterial proteins are converted into relatively harmless cleavage products before absorption takes place. The difference in toxicity on intravenous injection of dead bacteria which are subject to serum lysis and of those which are not, in the light of these findings, is probably not due to the greater concentration in the bacteria of toxic substances in the former case, but to an essentially extravascular digestion by leukocytes and other cells, and by fluids, of the

¹ *Folio Serologica*, 1911, 7, p. 243.

² *Ztschr. f. Immunitätsf.*, 1911, 11, p. 31.

³ *Berl. klin. Wchnschr.*, 1911, 48, p. 1072.

insoluble bacteria, lodged in the spleen or elsewhere, to a point beyond the toxic stage before absorption takes place. The greater toxicity of bacterial extracts prepared by grinding in the cold than of the corresponding number of dead bacteria may also be explained on this basis. For the same reasons, no doubt, intravascular infections are accompanied with severe clinical symptoms as compared with localized infections. In the former intoxication results during disintegration of bacteria, while in the latter the bacterial proteins are largely converted into nontoxic products before they are absorbed. This conception serves to explain also how large exudates, such as that in the lung in lobar pneumonia, may be absorbed without producing noticeable symptoms. That destruction of bacteria actually takes place during or preceding periods of intoxication of the patient in intravascular infections I have shown in two cases of chronic endocarditis.¹ Whenever the patient had a clinical reaction—chill, fever, and sweat—the number of viable bacteria in the blood dropped abruptly.

While toxic cleavage products are formed during disintegration of bacteria it does not follow that the bacterial proteins are the only source of toxic material during infections. The changes produced in the serum or other fluids during the growth of the bacteria might be a source of exactly similar toxic cleavage products. In order to throw some light on this point a study of the proteolytic changes, as shown by formalin titration, and the changes in toxicity which are produced in serum or other culture media during the growth of pneumococci, streptococci, and typhoid bacilli was made. The results show that protein splitting occurs with formation of toxic products before the bacteria give demonstrable evidence of disintegration. The amount of proteolysis in the culture fluid is greater than is obtained later by the complete digestion of the bacteria in NaCl solution. The symptoms and postmortem appearances are identical with those obtained by the injection of NaCl solution extracts. That this splitting may take place outside of the bacteria is certain, because proteolysis continues after the bacteria are removed by filtration. This and allied questions are at present being studied. This finding is in accord with

¹ *Jour. Infect. Dis.*, 1910, 7, p. 429.

what one could expect and points strongly in the direction that the mere growth of bacteria *in vivo* is probably an indirect but important source of toxic material. This may be another reason why intravascular infections are so severe.

The relation of these facts to the "endotoxin" theory may be discussed briefly. In the experiments, the results of which Pfeiffer¹ cites as proof that "endotoxin" exists preformed within certain bacteria, the toxic effects appear first in three or four hours after injection. The toxic action of bacterial extracts which are prepared without autolysis also appears some time after injection. The former result is not proof that the toxic action is not due to split products while the latter is good evidence that splitting must occur before the extracted bacterial substances can exert toxic effects. In my experiments in which proteolytic changes are proven to have taken place the toxic action occurs immediately after injection.

The great similarity in the symptoms produced by the injection at a certain period of the autolytic extracts of the various bacteria speaks in favor of certain common poisons for all; but it is very likely that substances which have a different chemical structure and are broken up into different toxic substances exist in the various species, one of which has the power to produce spasm of the unstripped muscle of the finer bronchioles in guinea-pigs. In this restricted sense it does not seem to be specific for the various bacteria. Other split products which may be assumed to have a molecular structure slightly different may have to do with the production of fever, still others with the paralysis of the vasomotor mechanism, etc. Differences in the symptoms produced during infections by the various species of bacteria may be explained among other things on differences in localization and the formation of different amounts of the various split products depending on differences in chemical composition, activity of autolytic ferments, etc., rather than on the assumption of the existence of a preformed specific "endotoxin" for each species.

CONCLUSIONS.

When the streptococcus pyogenes, the streptococcus mucosus, the meningococcus, the gonococcus, the typhoid bacillus, the

¹ *Jahrb. f. Immunitätsf.*, 1910, 6, p. 13.

colon bacillus, and the spirillum of Metchnikoff are suspended in NaCl solution the latter becomes very toxic at a certain period of autolysis. The time the acutely toxic substance appears in solution is directly proportionate to the activity of the autolytic ferment and the rate of disintegration of the microorganisms. In case of the staphylococcus aureus, in which little or no proteolysis took place, no toxic effect could be obtained with extracts in NaCl solution alone, but when treated with washed leukocytes or serum the toxic substance is readily brought in solution. Extracts of the pyocyanus bacillus, while toxic, never killed by producing symptoms resembling anaphylaxis.

As shown by formalin titration and by the polariscope, the anaphylatoxic substance from bacteria, just as has been shown to be the case with that derived from other proteins by other observers, is in all probability an early split product of the bacteria themselves.

The proteolytic enzymes contained in the bacterial cells are of importance in determining the degree and duration of intoxication.

The symptoms and postmortem appearances produced by the injection of the previously formed bacterial anaphylatoxic substance, whether by autolysis in NaCl solution, or by the action of leukocytes, or by treatment with immune and then with normal serum, or by a combination of these methods, either in the peritoneal cavity of guinea-pigs, or by the injection of filtrates of serum and serum broth cultures when proteolysis has reached a certain stage, and the immediate symptoms and changes following the second injection of protein in sensitized guinea-pigs, are identical. The acute death in each instance is asphyxia due to bronchial spasm.

The facts that bacteria, which are subject to serum lysis, are highly toxic before enough anaphylatoxic substance has been formed to kill by acute symptoms, and that, after this has been converted into harmless products, they no longer have a toxic action, speak strongly in favor of the view that the death in these cases is due to liberation of allied split products *in vivo*. The fact that death not infrequently occurs from two to 24 hours following an injection of a bacterial extract, which produces marked immediate symptoms, with symptoms wholly different, speaks in favor of the view that here death is due to other toxic cleavage products.

Intoxication during infections with bacteria which do not produce soluble toxins is probably produced by at least two distinct mechanisms. During the growth of the bacteria, albuminous fluids in which they multiply are converted into toxic cleavage products, while during their own disintegration they furnish similar toxic material.

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RAPID FILTRATION OF AGAR AND GELATIN.*

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In the ordinary routine work of a bacteriological laboratory the preparation and filtration of agar and gelatin is probably the most troublesome as well as the most tedious of any technic.

Since Koch revolutionized bacteriological methods, in 1880, by the introduction of the agar-agar culture media, which allowed the satisfactory isolation of organisms, as well as the preparation of stable solid media, workers have been endeavoring to find a more satisfactory and less tedious method of filtration. In many laboratories filtration is carried out by steam or hot water funnels and absorbent cotton, in others the funnel with the absorbent cotton or canton flannel along with the filtering flask is placed in flowing steam. Many do not even go to this trouble and are satisfied with filtering the hot medium through cotton at the room temperature. Drigalski¹ has described "Ein Schnell-filter für Agarlösungen," which is a device for the preparation of agar with the subsequent filtration of the medium in the same apparatus. It gives a large filtering surface and adds thereby to the rapidity of the process.

A method, which has apparently been overlooked, and which is not used in America, as far as the writer knows, was described by

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¹ *Centralbl. f. Bakt., I. Abt., Orig.*, 1906, 41, p. 2.

Bisserie¹ in 1907. The idea involved in his description has been used and modified in our laboratory, and has given us the utmost satisfaction.

After experimenting with many different processes, the simplicity, rapidity, and great efficiency of our modification over other methods has prompted us to bring forward our article. Our procedure in the preparation of 1,000 c.c. of agar is as follows:

A. DOUBLE STRENGTH BROTH.

1. Weigh out—

Peptone (Witte).....	10 gm.
Sodium chloride.....	5 “
Meat extract (Liebig's)	3 “

Add—Distilled water..... 500 c.c.

2. Mix together and thoroughly boil for half an hour in a double boiler. “As it is necessary to make the contents of the inner compartment boil, the temperature of the water in the outer compartment must be raised. This is done by using 25 per cent solution of common salt, which raises the boiling point 4.5° C.” (Stitt, 1910).

3. Titrate and correct reaction. For neutral agar make this double strength broth neutral. For 0.6+ agar, the broth must be 1.2+, or double the acidity desired in the finished medium. The agar solution, as prepared below, is slightly alkaline or neutral.

Boil again after titration to permit the complete precipitation of the phosphates. Retitrate to control result and to make certain of the stability of the acidity.

4. Filter through ordinary white filter paper. Make up to 500 c.c.

B. AGAR SOLUTION.

Agar-agar (for 1.5-2 per cent in finished product).....	15-20 gm.
Distilled water.....	500 c.c.

The agar should be finely divided and partly dissolved in the double boiler. (We have found the stick agar cleaner and better

¹ *Ann. de l'Inst. Pasteur*, 1907, 21, p. 235.

than either the thread or the powdered agar. Place the agar mass in the steam sterilizer and heat to 120°C . for a few minutes. This will give a perfect solution of the agar in the shortest possible time, being decidedly more rapid than when the agar is added directly to the ordinary broth. Moreover, the complete solution is an absolute necessity to insure the success of any method of filtration. Mix together A and B while both are still hot; boil together for a few minutes. Allow to cool to $55^{\circ}\text{--}60^{\circ}\text{C}$. Add the whites of two

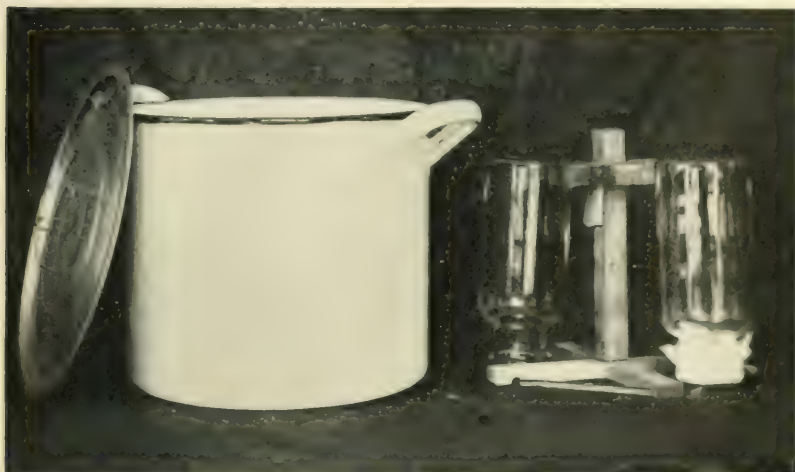


FIG. 1.

eggs well beaten in water to the agar preparation, mix thoroughly, and pour the mass into the filtering apparatus.

FILTERING APPARATUS.

The model as used by us is as follows (see Fig. 1):

1. An enameled pot 8.5 inches high by 8 inches in diameter, with a cover.
2. Four ordinary bottles 7 inches high by $2\frac{1}{2}$ inches wide, with a neck $1\frac{1}{2}$ –2 inches in diameter (not wider).
3. Canton flannel cut in convenient sizes to cover mouths of bottles.
4. A wooden appliance to steady the bottles when in position.
5. Small glass rods.

The cotton flannel is tied firmly over the necks of the glass bottles. The wooden apparatus is placed in the enameled pot containing the prepared nutrient agar. The bottles are put in position with the covered necks downward resting on the glass rods. The cover is put on the pot and the whole placed in the steam sterilizer, heated to 120° C. for a few minutes, to permit a firm coagulation of the egg, and then allowed to cool slowly.

The principle of this filtering method depends on the expansion of the air in the inverted bottles which, bubbling out during the heating, leaves a vacuum on subsequent cooling, and thus exerts a strong suction on the medium, which is slowly drawn into the bottles. The glass rods prevent the bottles from being drawn tightly to the bottom of the container, which would of course stop entirely all further filtration.

The agar prepared and filtered as above is perfectly clear, much whiter than ordinary agar and there is no clouding on subsequent sterilization. By preparing the agar medium in two distinct stages, so that the agar solution and broth are not repeatedly heated together, we find that the darkening of the medium is avoided.

Gelatin is prepared in the usual manner, and it is found that filtration is accomplished equally well by heating it to only 100° C. in the steam sterilizer sufficiently long for the thorough coagulation of the egg.

Glycerin jelly, for use in the mounting of pathological specimens, which offers exceptional difficulty in preparation, is also readily filtered by this method.

We have purposely excluded all metal from this apparatus, having found that small amounts of iron are sometimes absorbed by the hot media, giving color reactions with certain organisms which is undesirable.

The size of the filtering surface we have found to be of some importance. If the vessel has too wide a neck the filtering cover is drawn so far into the interior as to seriously interfere with the complete filtration. If the area is too small the egg may clog the pores of the flannel.

In using wider mouthed vessels, we have employed a perforated metallic disk over which the filtering cloth is tightly drawn.

Although this is quite satisfactory, as far as the filtration goes, we have, for the above reason, preferred not using metals of any kind in our apparatus.

The advantages of the above method are obvious. The final product, whether agar or gelatin, remains much lighter in color, and is devoid of the fine cloud or haziness so commonly obtained by the ordinary methods of filtration. Moreover, the method may be carried on with greater rapidity and with less personal attention. When the filtration is complete there is remarkably little residue. The simplicity of the method makes it adaptable to every laboratory, without the purchase of a special apparatus.

CHOLERA.*

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SIMPLE METHODS OF BACTERIOLOGICAL DIAGNOSIS.

In the examination of many specimens of feces for the presence of cholera vibrios, labor and time spent must be reduced to a minimum. This is especially evident in the examination of suspected carriers where it may be necessary to handle hundreds of cases a day. With such an amount of material even the best equipped laboratory would be crippled in attempting to carry out any but the simplest methods, especially in the face of an emergency. For reasons of expediency, the following simple method has been adopted after trying different methods suggested by other workers.

Collection of material.—Feces should be obtained whenever feasible and should reach the laboratory promptly, though if a large number of persons must be examined the collection of feces is almost impossible. The procedure adopted by Dr. Doty of taking rectal swabs is simpler and seems to be fairly reliable. These swabs are the ordinary wire, cotton-tipped swabs, such as are used in diphtheria work. They should be moistened with sterile peptone solution to facilitate introduction and prevent drying during transit, which might cause the death of the vibrios if there is any delay in reaching the laboratory. We have distributed the swabs with several drops of peptone solution in the bottom of the tube, against which the cotton rests.

Media.—Three media have been used, viz., Dunham's peptone water, Dieudonné's¹ blood agar, and alkaline 3 per cent agar. Not all are necessary in routine work.

Outline of method.—

Feces.—Direct examination.

Peptone water.—Incubate eight to 12 hours. Examine smears from surface growth. Examine those showing vibrios, in hanging

* Received for publication December 21, 1911.

¹ *Centralbl. f. Bakt.*, I, Orig., 1909, 50, p. 107.

drop, with and without agglutinating serum, making the hanging drops from the surface growth. If vibrios are too few in number for this or if smears are negative subinoculate into second peptone tubes.

Peptone water (subculture).—Incubate eight to 12 hours. Examine as above. Those showing no vibrios or too few to see in the hanging drop are considered negative.

Direct examination.—This is of no value except in acute cases. If a large number of typical and extremely motile vibrios are present, the case may be considered positive. The difficulty is that occasionally stools from cases other than cholera show rather numerous vibrios.

Peptone water.—There is nothing to be gained in examining hanging drops from tubes in which no vibrios are revealed by smear. Further, it is a waste of time to examine the smear too long, for if the tubes contain cholera vibrios sufficient for diagnosis they are very quickly found. On the other hand, if they are few in number, a second enrichment becomes necessary.

The surface growth in mixed cultures containing cholera is so nearly pure that on the addition of agglutinating serum the prompt clumping and loss of motility is sufficiently evident to be diagnostic. This application was suggested by Pollaci.¹ It is seldom that other vibrios enrich to this extent. If they do they can be excluded by the absence of any effect of the serum. When they are fewer in number, their motility may be like that of the cholera vibrio, which motility makes them evident even in hanging drops of mixed cultures. If the serum has no influence on this motility the vibrio can be excluded as not being cholera.

Subculture: peptone water.—A subculture in peptone water is necessary, as in four instances we have found cholera vibrios in subcultures where the first series of tubes were negative. The feces in these cases were not from cases showing clinical evidences of cholera. In the examination of acute cases no such necessity of further enrichment is likely to arise. In the examination of suspected carriers, however, an appreciable number of positive cases would be overlooked without a second enrichment. This

¹ *Deutsch. med. Wchnschr.*, 1911, 37, p. 354.

second enrichment helps to exclude the vibrios other than cholera. Some will have died out, others will not have enriched beyond the amount present in the first tube; these are not cholera. All vibrios which have enriched are then differentiated with agglutinating serum.

In examining for carriers the examination of the first peptone tubes may be dispensed with altogether. No fear need be entertained that cholera vibrios will be lost; as a matter of fact, they will enrich still further in the second tube. This delays the findings in some instances but becomes necessary where the number of examinations is large. In transferring the cultures, the first peptone tubes should be inspected, as positive tubes show a characteristic surface cloud. Any tubes showing this should be examined at once.

This method requires no elaborate equipment and allows the examination of large numbers of cases with the least expenditure of time. If selective media are used, such as the Dieudonné or the modification described in the next section, they should be used in duplicate with the peptone tubes. The great advantage of this method is its applicability to emergency work.

SELECTIVE MEDIA. A NEW AND SIMPLE MODIFICATION.

When Dieudonné prepared his alkaline blood agar, he was the first successfully to apply a fact known for years, viz., that the cholera vibrio could grow in the presence of alkali sufficient to inhibit other fecal bacteria. Deycke had attempted the use of alkaline albuminates in 1893 but without uniform results.

The formula of Dieudonné's medium as now in use is as follows:

- | | | |
|----|-----------------------|--------|
| A. | Defibrinated blood | } a.a. |
| | Normal sodium hydrate | |

Mix thoroughly and steam in the Arnold sterilizer for three-quarters of an hour.

B. Meat peptone agar, neutral to litmus, containing 3 per cent of agar.

Mix A, 30 parts, and B, 70 parts, while hot, and pour thick plates. The plates are then left open for 20 minutes to dry. They

are then placed in an oven at 50° to 60° C. for 20 to 30 minutes and finally left half open in the incubator overnight. If used before this treatment cholera itself will not grow on the plates.

Since Dieudonné others have added facts which tend to simplify the preparations of the medium. Pergola¹ found that the meat was not necessary in the agar, and that if this were left out the agar could be used without correcting the reaction. Recently Pilon² has substituted a 12 per cent crystalline sodium carbonate solution for the sodium hydrate. The plates can then be used at once; in fact, the blood mixture need not be heated if the plates are used immediately.

During our work with the Dieudonné medium it seemed to us susceptible of improvement in two particulars: first, if the medium could be rendered transparent; second, if a more easily obtainable material could be substituted for the blood. Where cholera examinations are of infrequent occurrence, or where blood is not easily obtained, such a change would be extremely important.

Experiments were then undertaken substituting eggs for the blood. Various mixtures were tried of the white alone, giving a transparent medium, and the whole egg, which gives a translucent medium. The following combinations were found satisfactory, in that they showed the requisite amount of restraint of the fecal bacteria with free growth of the cholera vibrio.

Egg-white medium.

- | | |
|---|-----------------------------|
| A. White of egg and water, a.a. | } Mix in equal parts, steam |
| Sodium carbonate cryst. 12 per cent. | |
| B. Meat peptone 3 per cent agar, neutral to litmus. | |

Whole-egg medium.

- | | |
|--|-----------------------------|
| A. Whole egg and water a.a. | } Mix in equal parts, steam |
| Sodium carbonate 12 to 13.5 per cent. | |
| B. Meat-free agar, viz., peptone, salt, and 3 per cent agar. | |

In either case mix A, 30 parts, and B, 70 parts, while the agar is boiling hot. Pour medium thick plates, allow them to stand

¹ *Centralbl. f. Bakt.*, I, Orig., 1911, 59, p. 83.

² *Ibid.*, 1911, 60, p. 330.

open for 20 to 30 minutes to dry, and then inoculate by surface streaking.

In making the egg mixtures, the water and egg must be thoroughly mixed, the alkali added and again well shaken. Filtration through a thin layer of cotton may be employed to remove any of the thicker parts of the egg.

Of the two formulae, the whole egg gives the more vigorous growth of vibrios, requires no meat in the agar, and is more economical. The egg white, on the other hand, requires a meat agar, the growth is not as vigorous, and crystals frequently form, especially along the inoculation streaks. Its only advantage is its transparency. For general use, therefore, the whole-egg mixture is better. Comparing the whole-egg medium with the blood medium of Dieudonné, the latter is somewhat more selective; that is, it seems to restrain the common fecal bacteria somewhat more. This, however, is more than offset by the fact that should other bacteria grow the cholera colonies on the egg medium are distinctive. Examined by transmitted light they have the appearance of being deep in the agar and have a peculiar hazy look, due to a halo about the colony. Where the growth is very vigorous this halo is surrounded by a zone of clearing. In practice, fishing from this type of colony has yielded cholera and from this type alone. The only exception is in the case of cholera-like vibrios, their colonies being identical. The plates keep when stored on ice for a limited time. The egg alkali mixture seems less permanent. This is unimportant compared with the more permanent Dieudonné's blood mixture, as the egg mixture can be prepared so quickly.

Because of the ease of preparation and the prompt availability of the ingredients this medium is an improvement, especially for those who are called upon to examine cholera-suspected stools at infrequent intervals. When one considers that 10 eggs is sufficient for over 300 plates the medium is economical even where large numbers of examinations are being made. Furthermore, it is an improvement for the reason that cholera grows in distinctive colonies, allowing quick selection for smears and agglutination.

CHOLERA LIKE VIBRIOS.

The vibrios other than cholera found in stools are of little practical importance. When, however, the present trouble arose there was no cholera agglutinating serum available for immediate use. Under these circumstances the detention of a ship was necessary because of the presence of a cholera-like vibrio in one case. This is an emergency that is likely to arise at any time in a country where cholera examinations have not been required for many years.

Because of this we have tested the various vibrio strains we have isolated, to see if there were any biological peculiarities which would quickly exclude those not cholera. This has yielded some results. Some of the vibrios can be excluded because of the marked tenacious pellicle they form on peptone, or the dry adherent growth on agar. Of 50 vibrios other than cholera, 43 did not give the cholera-red reaction.¹ Of the seven that did, two did not produce acid, and four produced gas in litmus-glucose peptone, only one produced acid like cholera, but this strain formed a tough pellicle and was adherent on agar. These differences were evident in less than 24 hours' incubation.

If, then, a colony be fished and inoculated into peptone water, glucose media, and agar, many, if not the majority, of vibrios other than cholera could be quickly excluded.

The ability to liquefy Loeffler's blood serum and gelatin and the fermentation of saccharose and lactose as well as milk were tried. The acid change in lactose, milk, and the liquefaction of gelatin and Loeffler as exhibited by cholera is too slow to be of practical use. The fermentation of saccharose is more prompt. About half of the other vibrios ferment this sugar, some with the production of gas.

A few vibrios we have found to be only slightly motile. When mixed cultures containing vibrios are subinoculated in peptone water, they do not show the same ability to enrich as shown by cholera. Many enrich to a limited degree and then remain con-

¹ Care should be used in determining this, as in several instances we got a reaction with peptone water to which sodium hydrate had been added to increase the alkalinity, not otherwise. Whether this was due to traces of nitrate we cannot say.

stant. Others enrich slightly and die out in the third tube of peptone. This may be considered evidence that they are not cholera.

HEMOLYTIC ACTION OF CHOLERA AND ALLIED VIBRIOS.

Because of the number of varied reports that have appeared on the subject of hemolysis by cholera and other vibrios, we have tested 50 cholera and 50 non-cholera strains for their hemolytic properties. These were recently isolated and included one culture from the Philippines and two from Italy. One old culture isolated by Buxton in 1893 and one El Tor strain were also tested. This latter strain is one of the six isolated in 1905 by Gottshlich at El Tor from the cadavers of pilgrims to Mecca. These pilgrims had died with diarrheal symptoms, but had shown no clinical evidences of cholera. The El Tor strains agglutinated like true cholera, but were strongly hemolytic. The hemolytic power was considered by some, especially Kraus and Ruffer, to be sufficient evidence for regarding these vibrios as not true cholera. This was based on Kraus' statement that the hemolytic power was not a property of true cholera, but was only found in allied types.

Our results were as follows:

Thirteen non-cholera vibrios.....	weakly hemolytic.
Four non-cholera vibrios.....	strongly hemolytic.
El Tor.....	strongly hemolytic.
Cholera, Buxton. ¹	strongly hemolytic.

The following technic was employed: to 1 c.c. of a suspension of washed sheep, goat, or horse corpuscles, was added .25 c.c. of a week-old broth culture. Readings were made after two and three hours' incubation. The tubes were then shaken and put in ice for 24 hours, when final readings were taken.

Our results with the cholera vibrios agree with those of Mühlens, von Raven, Neufeld, and Haendel, and others who have found that laboratory cultures of true cholera may possess or acquire hemolytic power.

¹This strain showed cultural variations. It did not liquefy Loeffler's blood serum and was extremely slow to liquefy gelatin. We could not test its agglutinability, as it made a poor suspension which quickly flaked out. In rabbits, however, it produced agglutinins for true cholera.

On the other hand, Mühlens and von Raven found that three rather recently isolated strains possessed hemolytic powers. Baerthlein¹ also found some newly isolated strains strongly or weakly hemolytic, depending upon the technic used. When he inoculated corpuscle suspensions from agar slants and incubated 24 hours or longer, he found one strain strongly, and others irregularly, hemolytic. When he used a five-day broth culture, the same strain showed comparatively prompt hemolysis. All, however, showed varying degrees of hemolysis at 48 to 72 hours. With blood agar plates again only the one strain showed hemolysis.

Although the technic of different observers has differed so greatly as to make it difficult to correlate the results, there can be no doubt that there is great variability in the hemolytic powers on successive tests even of the El Tor cultures. Freshly isolated cholera cultures do not possess strong hemolytic powers, though this property may be acquired after artificial cultivation for longer or shorter periods of time. In a few cases freshly isolated strains have been strongly hemolytic or have shown traces of this capacity. This capacity may not be as well developed as in some non-cholera vibrios, but it cannot be considered of sufficient importance to justify the separation of strains biologically true cholera but hemolytic, from non-hemolytic strains. If a strain, El Tor or other vibrio, agglutinates in a cholera-immune serum, it must be considered cholera, whether it hemolyzes or not. The fact that the El Tor strains were isolated from pilgrims who must have become infected months previous to their death, that is, were carriers for an unusually long period, and who, during the pilgrimage, were not the source of cases of cholera, is none the less remarkable than that each strain isolated should be the same in possessing hemolytic properties.

¹ *Arb. a.d.k. Gsundheitsamte.*, 1911, 36, p. 446.

ON PLASMA CELLS IN THE TONSILS.*

DAVID J. DAVIS.

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The literature on the subject of plasma cells is very extensive and much discussion on the morphology, significance, occurrence, identity, function, etc., of this cell has appeared. Many problems concerning it are unsolved. However, it is generally agreed by most observers, both from experimentation and otherwise, that the plasma cell is a well-defined cell, that it occurs under fairly definite though manifold conditions, and like all other cells is subject to various degenerations, which often give rise to atypical morphological appearances. There are also transitional forms of cells undoubtedly closely related to it. It should be looked on as a cell as clearly defined as, for instance, the polymorphonuclear leukocyte, or the connective tissue cell.

This cell was described definitely first and named by Unna in 1891, and, to be brief, has these characteristics: a roundish oval cell, 6 to 16 μ in diameter, the protoplasm of which stains characteristically by basic dyes (especially pyronin and polychrome methylene blue) and more deeply at the periphery than about the nucleus. Its nucleus is generally eccentrically placed and possesses a characteristic peripheral clumping of chromatin into five to eight definite masses. To these characteristics should be added the presence of the Altmann-Schridde granules in the lighter staining protoplasmic zone immediately about the nucleus.

The origin of these cells has been a much disputed question. Unna thought they came from the connective tissue cells. While this idea is still supported by a few observers, most recent workers now consider them as derived from the small lymphocytes. This view though not original with Schridde has been greatly strengthened if not absolutely proved by his demonstration that the characteristic Altmann-Schridde granules appearing in the protoplasm of plasma cells appear also in the small lymphocytes and in the

* Received for publication November 25, 1911

large cells in the centers of lymph follicles. As they are not known to occur in other cells their presence in these three varieties suggest a close relationship. A discussion of the significance of plasma cells will be considered in the latter part of the paper.

This report is based on observations made on about 240 pairs of tonsils; 180 were extirpated chiefly because of hypertrophy, and were mostly from children between five and 15 years. There were many, however, from young adults who had suffered from recurrent tonsilitis, rheumatism, nephritis, etc. The remainder, about 60 in number, were obtained at autopsies from subjects of various ages, ranging from the fetus to the very aged;¹ seventeen were infants less than three months old. With few exceptions the tonsils from autopsies appeared normal. As a routine method of staining the methyl-green-pyronin stain of Pappenheim was used.

Concerning the occurrence of plasma cells in the tonsils the following facts were noted: They are not found in the fetus or in the newborn child. They make their appearance in the tonsils with more or less regularity about the second and third week of life. Some variability in this respect exists. In one case, for instance, during the second week a few were found; in another case, at the end of the third week none was found; in still another, at the end of the eighth week none was found. In no case examined older than this were they absent. At first very few are found and they are seen as a rule just beneath the epithelium lining the crypts. They increase in number gradually, so that in children several months old they occur as a rule constantly and abundantly. They remain present throughout life, even to very old age (88 years the oldest examined), regardless of the anatomic condition of the tonsil. In pathological tonsils, and especially in hypertrophy, they are far more numerous.

As to their distribution in the tonsil, they occur chiefly in three localities. They are most numerous just beneath the epithelium, especially that lining the crypts. In the papillae they are often seen in large numbers about the small blood vessels.

¹ Much of this postmortem material was obtained at the Pathological Institute at Freiburg i. Br. Director Professor Aschoff, to whom I wish here to acknowledge my indebtedness for many favors.

Beneath the surface epithelium they are found, but generally not in abundance. Often in the epithelial layers they may be seen in the act of migration, and when thus found are usually single or in twos. They may be free in the crypts but as a rule in very small numbers.¹ A second locality where they are found abundantly is along and in the meshes of the connective tissue that surrounds and penetrates the tonsil. Here they occur singly or in loose clusters and are often associated with mast cells, which are always far less abundant. Thirdly, they often but by no means always may be seen clustered about the smaller blood vessels at various depths in the lymphoid tissue. In addition to these localities occasional plasma cells may be seen in the lymphoid tissues, though it is somewhat surprising how rare they are here. It is possible, of course, that such isolated cells may be near a small blood vessel located outside the section. I have never in any case observed a typical plasma cell in the center of a lymph follicle. They often occupy lymph spaces, especially the perivascular lymphatics, though they are not limited to such vessels. Apparently they wander through the various tissues as they do through the epithelium into the crypts. In hypertrophied tonsils their distribution is not different from that in normal tonsils. As was stated, they are more numerous and I think their number is roughly proportional, in a general way, to the increase in size. In such cases they are seen in clusters or often in a continuous layer under the epithelium or about blood vessels. A not inconsiderable part of the increase in size of such tonsils is evidently due to the accumulation of the plasma cells.

In connection with the facts concerning the time of the appearance of plasma cells in tonsils I made some observations with the view of determining at what time the tonsillar crypts are invaded by bacteria. As is well known, tonsils from the fetus and newborn are sterile. Cultures made during the first week and later reveal bacteria, as do also stained preparations of the tonsils. These bacteria are a mixture of various bacilli and pyogenic cocci. Evi-

¹ It should be stated that plasma cell-emigration seen in the epithelium of the tonsils is entirely different from the lymphocyte-emigration described years ago by Stöhr, who thought the lymphocytes passed through the epithelium, leaving openings (physiological wounds) through which bacteria might enter.

dently, then, as one would expect, invasion of the tonsillar crypts occurs at about the same time as, or shortly after, the intestinal canal is invaded by bacteria, which is, as has been shown by others, from 24 to 72 hours after birth.

It thus appears that soon after invasion of the tonsils by bacteria the plasma cells appear in these organs. The question at once arises as to whether these two phenomena are in any way related and whether the one is dependent on the other. This problem perhaps can not be decided definitely, but it may be worth while to make a few statements which may have some bearing on this point.

Plasma cells are found chiefly in connection with pathological lesions, especially those of a chronic nature (granulomats). In acute infections they are not numerous. But in chronic infections they are present, usually in enormous numbers, and in certain conditions, as, for example, in gonorrheal salpingitis, their numbers and location may be sufficiently characteristic to be of aid in diagnosis (Schridde, Amersbach). In some skin diseases, as in lupus, mycosis fungoides, and some others, they accumulate in large masses in the lesions. They are known to occur constantly in the gastrointestinal mucosa, and J. Schaffer¹ has observed them in the involuting thymus in man, the mole, mouse, dog, and white rat. They are found commonly in tumors, especially at the margin of carcinomats. Tumors have been described (Wright, Aschoff, Hoffmann, Christian), occurring chiefly in the bone marrow, which are composed almost entirely of plasma cells (plasmocytoma). These tumors do not produce metastases, and therefore by some are considered in the group of chronic infectious granulomats.

Experimentally it has been shown by a number of observers² that extensive accumulations of plasma cells occur in animals following the injection of various bacteria. This is true especially for Friedländer's bacillus and the gonococcus. The introduction subcutaneously or intraperitoneally of irritating nonbacterial substances, such as turpentine and tissue extracts, also gives rise to an accumulation of these cells.

¹ *Centralbl. f. Phys.*, 1909, 22, p. 858.

² *Centralbl. f. allg. Path. u. path. Anat.*, 1909, 20, p. 1011.

A careful and systematic study of these cells has apparently not been made in the tissue of the fetus and the newborn. Schridde¹ states that only in later life are plasma cells found in the spleen and are to be regarded as pathological. Aschoff has observed that in the appendix in the newborn, plasma cells are not present. Soon after birth they appear and are thereafter found constantly in all appendices, normal and pathological. In lymph glands they occur commonly, especially in glands undergoing fibrosis. I have seen them in enlarged glands in Hodgkin's disease but not in large numbers. I can find no statement concerning their occurrence in lymph glands of the fetus or newborn. Such glands examined by me in a few cases, revealed no plasma cells. Porcile² observed plasma cells in the livers of two newborn children, one having syphilis of the liver, the other syphilis of the lung.

In general, then, these cells may be said to occur chiefly in chronic inflammatory lesions, along the gastro-intestinal canal and in connection with certain atrophic processes. Now in view of the fact that plasma cells occur in such large numbers in tonsils, appendix, and some other tissues after birth at a time when the crypts and intestinal canal are infected, and associating this with the fact that in chronic infections generally the plasma cell is so constantly found, one is led to suggest that their presence in the tonsil and appendix, and also in the gastro-intestinal tract, lymph glands, and spleen is due to a chronic infectious or absorptive process. In such organs as the tonsil and the appendix, whose crypts or lumen are constantly infected by many organisms, often, as has been shown, of a pathogenic nature, absorption of bacteria or toxic products must occur to some extent. This is also true throughout the intestinal canal, as it is well known that bacteria are constantly passing through the wall into the lymphatics and blood stream. Indeed, Dantchakow and Pirone,³ after noting the occurrence of these cells especially in the intestinal mucosa in atrophic organs like the thymus and following the injection of tissue extracts, suggested that the absorption of ferments caused sufficient irritation

¹ *Pathologische Anatomie*. L. Aschoff., 2, p. 116.

² *Beitr. z. path. Anat. u. z. allg. Path.*, 1904, 36, p. 375.

³ *Centralbl. f. allg. Path. u. path. Anat.*, 1909, 20, p. 1011.

to promote the accumulation of plasma cells. This may be entirely or only partially true.

In the case of the tonsil, however, the view is more rational that this organ is, in all cases, whether hypertrophied or not, a chronically infected focus from a time shortly after birth, a view apparently supported by bacteriological studies. It therefore seems reasonable that cells more or less characteristic of chronic inflammation should accumulate here. Not only the nature of these cells, moreover, but their accumulation in such large numbers and in localities immediately underneath the epithelium, especially about the infected crypts, also favors such a view. Similar conditions evidently exist in the appendix, another organ which is so often subject to severe infections.

SUMMARY.

In the tonsils of the fetus and the newborn, plasma cells are not present. They first appear about the second or third week, and thereafter are constantly found in the tonsils. This is about the time, or shortly after the time, that bacteria invade the tonsillar crypts. In hypertrophied tonsils they are more numerous than in apparently normal tonsils. Their presence may be interpreted as indicating the existence in the tonsils of a chronic infectious process or the absorption of toxic or irritating products.

BACTERIOLOGY AND PATHOLOGY OF THE TONSILS
WITH ESPECIAL REFERENCE TO CHRONIC
ARTICULAR, RENAL, AND CARDIAC
LESIONS.*

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The relation of tonsillar infection to lesions existing in other regions, especially in the joints, heart, and kidneys, has received much attention, particularly from the clinical side. That a causal relationship not infrequently exists appears to be well recognized and is apparently based chiefly on the fact that often after tonsillectomy, in cases where there is reason to believe that such a causal relationship does exist, marked benefit or permanent cure follows. The bacteriological work that has been done in such cases is in many respects incomplete and unsatisfactory and in the study of cases of this nature, begun about three years ago, it appeared to be necessary in the first place to take up this phase of the problem with especial reference to organisms of the streptococcus and related groups.

Our knowledge of the bacteria of the tonsils has very largely been obtained from the study of swab cultures and smears made directly from the surface of the tonsils *in situ*. This surface is necessarily contaminated by bacteria from the mouth, throat, nose, food, inspired air, etc., and such examinations may not only give very incorrect data about the bacteria that actually exist in the depths of the tonsils but also may at least partly explain the reason why such a complex bacterial flora has been attributed to this organ.

Very early in this work, from a comparative study of the superficial and deeper flora in a number of cases, I became aware of the fact that swab surface cultures were of little value, because they revealed little or nothing about the bacteria in the crypts. Inasmuch as the latter are of far greater importance than the

* Received for publication January 15, 1912.

surface flora it became necessary to make careful cultures from the depths of the crypts as well as from the surface. The following technic in the examination of extirpated tonsils was therefore used: the excised tonsil was received in a clean receptacle or in clean gauze and, as soon as possible, smears and blood-agar plate cultures were made from the surface. The tonsil was then incised with a hot knife and from the crypts thus exposed similar smears and cultures were made. In many cases, for control, swab cultures of the surface were made before extirpation and compared with those made after.

The extirpated tonsils from 113 cases have been examined bacteriologically. Many more were examined grossly or microscopically, but for various reasons were not suitable for bacteriological work. The tonsils, usually more or less hypertrophied, were obtained from cases of joint lesions, nephritis, heart disease, tonsilitis, and some were examined also from cases of simple hypertrophy without serious complications. These conditions will be discussed separately.¹

ARTHRITIS.

In this group are 28 cases, which include a variety of conditions ranging from simple articular pains associated with tonsilitis to severe chronic involvement. Some gave histories of having had typical attacks of acute articular rheumatism, but usually the arthritis was chronic in character with frequent more or less acute exacerbations. In some instances the arthritis was deforming and associated at times with exudates into the joints.

From the crypts hemolytic streptococci were obtained as the predominating organism in 25 cases. In many they were in pure

¹ In discussing the bacteriology of these cases reference will be made chiefly to the predominating organisms on the plate culture. Manifestly occasional colonies of various organisms that have no importance whatever will appear on plates made under such conditions. These include moulds, chromogenic bacteria, white staphylococci, colon bacilli and the like. Such bacteria occurring sparsely and with no constancy on the plates were considered of no import and discarded. In anaerobic cultures made in a number of the cases the number and variety of bacteria obtained have been few and inconstant. It should be stated that in smears one not infrequently sees bacilli, often in considerable number, which so far have not been cultivated. More might be said about such organisms, but here it is sufficient to state that there is no reason to believe that they possess any pathologic significance. The term "pneumococcus" in this paper includes organisms which produce a green zone on blood-agar plates and which occur generally in diplococcus form. Many of these organisms grow in long chains; some ferment inulin, and others do not.

growth and in large numbers. In two cases the pneumococcus predominated, though a few hemolytic streptococci were present also. In the remaining case the streptococcus mucosus was found, together with a few hemolytic streptococci. It will thus be seen that hemolytic streptococci were found in every case in the tonsils and predominated in nearly all.

Rabbits were inoculated intravenously with streptococci from 17 of the joint cases. All but two strains caused lesions in the animals and in one instance, associated with the arthritis, was endocarditis of the tricuspid valve. Pneumococci from five cases, including the two in which they were predominant, were introduced into the veins of rabbits and in one animal non-fatal monoarticular arthritis developed.

Three of the cases of arthritis were complicated by chorea, either at the time of examination or sometime previously. They all revealed a predominant streptococcus flora in the tonsillar crypts, and upon injection of two of the strains into rabbits both developed acute multiple arthritis. Neither chorea nor endocarditis was noted in these animals.

In but two individuals suffering with severe chronic arthritis was it possible to obtain fluid from the joints. In both cases the fluid was straw-colored, slightly turbid, and some fibrin formed on standing. Polynuclear leukocytes were present but not in large numbers. The fluid and also the blood in these cases were sterile.

NEPHRITIS.

These cases, 10 in number, were chiefly chronic, and many had developed nephritis shortly after having had colds or tonsillitis. Some were mild, others very severe, and two were hemorrhagic in character. Two were complicated by arthritis. The tonsils as a rule were enlarged and the surfaces uneven and usually red. In nine of the 10 cases hemolytic streptococci were found predominating or pure in the tonsillar crypts. In some a few pneumococcus-like organisms appeared but were in no instance in large numbers. In the one case, which did not reveal streptococci, a small aerobic, gram-positive, non-liquefying, non-hemolytic bacillus was found nearly pure in the crypts. This bacillus did not produce gas in

glucose, caused no change in milk, was non-motile, and i.c.c. killed a guinea-pig in 24 hours. It was not identified.

Streptococci from eight of the 10 cases were injected intravenously into rabbits and in all localization in joints or tendon sheaths occurred. The kidneys in these animals were examined with negative results. In the urine from a number of these animals there was no evidence of abnormal constituents and no streptococci were found.

ENDOCARDITIS.

There were 10 cases of endocarditis, nearly all of which were, at the time, suffering from arthritis or gave a history of having had it in the past. They were as a rule not severe, excepting two, which were malignant in character.

Hemolytic streptococci were found in six cases in large numbers and predominatingly. In the remaining cases were large numbers of pneumococci and in two these organisms were nearly pure. In one case of fatal malignant endocarditis with pneumococci in the blood and in the pleural and pericardial exudates the tonsils were only slightly enlarged, but near the base they were very fibrous, and in the depths contained small pockets filled with pus cells and diplococci. In the cultures from the crypts pneumococci grew almost pure, there being only an occasional streptococcus colony. Streptococci from four cases were injected into rabbits and arthritis developed in all. The pneumococci from three of the cases of endocarditis were injected into rabbits and vegetative endocarditis developed in two instances; no joint lesions.

TONSILLAR HYPERTROPHY.

The tonsils were examined from 61 cases of tonsillar hypertrophy, nearly all of whom gave histories of repeated attacks of tonsilitis. In 50 the hemolytic streptococcus was obtained as the predominant organism and, as in the other groups, in many instances the cultures were practically pure. In only two were pneumococci predominant, and in these some streptococci were also found. In one case the streptococcus mucosus was found in considerable numbers and in six cases the white staphylococcus

was the most numerous organism. In three cases the influenza bacillus was found predominant and on the blood-agar plates they appeared in enormous numbers in symbiosis with the few hemolytic streptococci also present. These are the only three instances in the entire series in which the influenza bacillus was found in the tonsillar crypts. Two of these three strains were tested on guinea-pigs and intraperitoneal injections of two blood-agar slant growths killed the animals in 24 hours; one slant-agar growth was not sufficient, as a rule, to cause death. In two cases typical diphtheria bacilli were found nearly pure in the crypts. In one case of recurrent tonsillitis, the last attack being four weeks previous to the tonsillectomy, cultures from the crypts gave a nearly pure growth of this organism, while cultures from the surface both before and after extirpation did not reveal it. This bacillus was fatal to a guinea-pig by intraperitoneal inoculation. There is some reason to believe that this case was responsible for an outbreak of diphtheria in a number of persons closely associated with this individual. In the second case, while the bacilli were typical morphologically, they were not pathogenic for guinea-pigs and therefore should be classed in the pseudodiphtheria group. In one case from this group a nearly pure growth of *B. mucosus capsulatus* was found in the crypts. Not infrequently an isolated colony of this bacillus appeared on the plate cultures but not in predominating or significant numbers.

Seven strains of streptococci from the cases of recurring tonsillitis were tested on rabbits and in every instance the animal developed arthritis in every way similar to that produced by streptococci from the other clinical groups. Four strains of pneumococci from these cases including the strains which predominated in two cases were injected into rabbits with negative results. In one remarkable case of multiple neuritis without arthritis or heart lesions, in the crypts of the hypertrophied tonsils was a nearly pure growth of hemolytic streptococci which produced a fatal multiple arthritis in rabbits.

The arthritis which develops in the animals injected with streptococci is manifested by the occurrence of lameness and swelling

of the joints and usually appears from the third to the fifth day after inoculation. If too large doses are given the animal may die of septicemia in 24 or 48 hours, but even this early the joints may be involved, as shown by redness of the lining membranes and the presence in the cavity of a few leukocytes and streptococci. The most suitable dose is the 24-hour growth from the surface of one small blood-agar slant. On the whole the strains isolated from the various cases are quite uniform in their pathogenic properties, a given dose in animals of uniform size usually producing similar results. Of far greater importance is the size and age of the animal. Large full-grown or old animals are resistant and indeed may withstand enormous doses (several slant-agar growths); on the other hand, young animals are so susceptible that even very small doses may give rise to septicemia and death in 24 hours or less without definite localization of the infection. Half to three-quarter grown animals are most suitable for this work.

Some of the animals die in the course of one to three weeks, but in many the joint lesions gradually subside and the animal recovers. In the less severe cases few joints or only one may be isolated, but usually the arthritis is multiple and in some of the rabbits almost every joint in the body was attacked, including the vertebral joints. The carpal joints and the joints of the hind limbs are often the seat of localization. In a number of animals the process became chronic, the joints increasing in size with marked destruction of bone and joint surfaces. In one case subluxation occurred after six weeks. Chronic joint abscesses may arise in which streptococci in few numbers may be found in the glary mucoid or mucopurulent contents, and such animals often live for months, gradually becoming very emaciated and badly crippled, but nevertheless appearing quite lively and eating normally. Ultimately most of them die, but usually without a septicemia. Streptococci may be found in few numbers in the chronic lesions, though in some instances the cultures were negative. In four animals, following non-fatal injections, the joints simulated a deforming arthritis. They became large and exostoses appeared about the ends of the bone. After several months the deformities in these cases gradually and apparently entirely disappeared.

The pathogenicity of a number of the streptococci was tested further by injecting them intraperitoneally into guinea-pigs. It was found that one culture almost invariably sufficed to kill by peritonitis in 24-48 hours.

A monkey (*macacus rhesus*) was given an intracardiac injection of a strain of streptococcus which had produced arthritis in a rabbit and been reisolated from one of the joints of the animal. After a few days multiple arthritis involving many joints on both fore and hind limbs appeared and death followed in about two weeks. The joints contained an abundant purulent exudate from which streptococci were cultivated. The heart's blood also contained them in moderate numbers, but there was no sign of endocarditis and no localization in any other part of the body.

Post-mortem examination of the animals revealed the fact that not infrequently in the early stages the involvement was periarticular and often extended to the tendon sheaths. At times, especially in the hind limbs, a tendon sheath might be involved throughout its entire length and the joint cavities remain entirely free.

Microscopic examination of sections through the extremities have been made in some of the animals. This work is being carried on at present in a systematic way in order to examine the joints at definite intervals following the inoculation, but the results are not yet complete. It would appear from the data at hand that the organisms first localize in the subserous structures about the joints, forming small infected foci, and from here secondarily break into the cavities.

The exudate in the joints early is glary and mucoid and soon becomes purulent, containing large numbers of polynuclear cells and streptococci. Usually abundant phagocytosis of this organism in the exudate is noted. After a week or longer, in some rabbits but not in all, the exudate may become thick and pasty. Streptococci can ordinarily be readily grown from these joint exudates, but a few exceptions have been noted. In one animal that died on the fifth day with definite arthritis, the cocci could be seen in the leukocytes, but careful cultures on blood media gave no growth. In two other instances though the involvement of the joint was unmistakable no organisms grew in the cultures.

In the bodies of the inoculated animals careful search was made for other lesions, especially endocarditis. Following streptococcus injections in only one instance was a lesion found, vegetative in character on the tricuspid valve. This was not extensive but was definite, and was produced by a strain isolated originally from the tonsillar crypts in a case of multiple arthritis. The joints of the animal were also involved but there was no other localization. The organisms were recovered from the heart's blood. Pericarditis was not found in any instance.

As stated above, in two instances pneumococci produced an endocarditis. In one case, by intracardiac injection, a very extensive mitral endocarditis developed in an animal, associated with large renal infarcts and septicemia but without joint lesions. This organism, which fermented inulin and gave the typical green zone on blood agar, was isolated from the depth of a tonsil in a case of severe multiple arthritis, not associated at the time, so far as could be determined clinically, with heart lesions. In this case also streptococci were abundant in the tonsils and upon intravenous inoculation into an animal produced arthritis. In the second case a definite but less extensive mitral endocarditis was produced by intravenous injection with pneumococci from the depths of the tonsils in a case of recurrent tonsilitis associated with endocarditis. From the valvular lesions the organism was recovered pure. It did not, even in huge doses, produce arthritis in animals.

Some of the strains of pneumococci were injected into animals in enormous doses without effect. This was true even in those instances where endocarditis did develop and is in marked contrast with the fact that streptococci produce arthritis in very much smaller doses. In only one instance with a pneumococcus-like organism did a joint lesion develop and this was not fatal.

Observations were made with the view of determining possible differences in the various strains of streptococci isolated from the clinical groups. On blood-agar plates these streptococci are alike, in that they are all hemolytic. The width of the zone of hemolysis may vary considerably. I have noted at times even on the same plate two kinds of hemolytic streptococci. The one possesses a wide distinct zone of hemolysis with a rather large gray central

colony, and is the ordinary typical streptococcus pyogenes; the other, met with only occasionally, has a much narrower zone, which may be slightly turbid, and the margin is less distinct and the central colony smaller. The difference is sufficient to enable one to separate them clearly, especially when they occur on the same plate. The latter organism forms as a rule shorter chains but shows no tendency to occur in pairs in the chain. It grows less luxuriantly in subcultures, and tends to die out more quickly. It produces typical arthritis on injection into rabbits but less readily than the more hemolytic variety, and on sugars they cannot be differentiated. It does not ferment inulin. It is surely not a pneumococcus, never producing a green zone on blood plates and not possessing the lanceolate form. After passage through an animal it still retains its properties. I am inclined to consider it a less virulent form of the ordinary hemolytic streptococcus.

Forty-three strains from the crypts of the tonsils from the various clinical groups were tested on dextrose, lactose, mannite, raffinose, and inulin, the acidity being determined after five days by titration with NaOH. Without going into minute details it will be sufficient to state that all produced acid in dextrose and none in raffinose and inulin. All but five fermented lactose, one of these strains coming from a case of arthritis, the other four from cases of enlarged tonsils with recurring tonsilitis. Eleven strains fermented mannite and 32 did not. The five strains which failed to ferment lactose failed to ferment mannite also. The grouping into mannite and non-mannite fermenters has no evident relation whatever to the clinical condition. For instance, some streptococci isolated from the arthritis cases fermented mannite and others did not. This applies also to the other clinical groups. Compared with streptococci from other sources (puerperal fever, erysipelas, empyema, scarlet fever), they show no morphologic or cultural differences. Five strains of pneumococci were tested on sugars. Two fermented inulin and three did not. The latter three include the two strains that produced endocarditis in the animals. Otherwise they behaved alike, fermenting dextrose and lactose but not mannite or raffinose. They rapidly acidify and coagulate milk, especially if the milk is mixed with equal parts of broth.

THE MICROSCOPIC EXAMINATION OF THE TONSILS.

The tonsils generally were moderately hypertrophied with often red and smooth or coarsely lobulated surfaces. In some instances they were very large, the crypts deep and tortuous, and at times contained yellowish caseous masses. Many, especially those from persons giving a history of repeated tonsilitis for a long time, were very fibrous, particularly at the base, and the crypts were much distorted.

Microscopically the most striking feature was the common occurrence of polynuclear leukocyte clusters far down at the end of the branching crypts. In some instances these appeared as if they were in the tonsillar tissue beneath the epithelium, but this was not true. The epithelium, especially in the hypertrophied tonsils, often along the course of the crypts becomes very thin and permeated with lymphoid cells. The two layers of epithelium come in close apposition and as a thin layer penetrate far into the depths of the tonsils. Often at the farthest extremity of such structures or possibly along the course or in a small lateral crypt one may find small clusters of polymorphonuclear cells, among which by proper staining methods bacteria, especially streptococci, may be found. The difficulty of proper drainage of such foci can well be appreciated.

Not uncommonly one finds, deep in the tonsil cavities containing bacteria, cholesterin crystals, hyalin material, and cellular débris and lined by epithelium. Occasionally similar structures are seen in which the lining epithelium has entirely disappeared and the connective tissue cells may be observed penetrating the mass while about the cholesterin crystals large multinucleated giant cells appear. Such cavities have undoubtedly been formed by adhesions or constrictions at the outlet of the crypts preventing drainage, and should pathogenic bacteria be present in such foci it can readily be understood how an abscess might form. I have observed in the series four small abscesses deep in the tonsils near the capsule, which were filled with thick pus and surrounded by a dense fibrous wall; the contents of three of these were sterile, no organisms being found either in smears or culture. In the adjacent crypts, however, in each case were myriads of streptococci. The abscesses were

undoubtedly present for a long time and the organisms which initiated the infection later in all probability died. In the one case in which the purulent contents was not sterile a mixture of streptococci and staphylococci were found.

There appears to be little or no difference in the lesions of the tonsils obtained from the various clinical groups. For example the recurrent tonsilitis cases may present changes identical with those seen in the articular, cardiac, or renal cases. In general the more numerous the attacks of tonsillar trouble have been the more extensive is the fibrosis and the more distorted are the crypts.

In all tonsils from individuals who are more than a few weeks old plasma cells are found in abundance. They occur under the epithelium, especially that lining the crypts, along the connective tissue bundles and about the blood vessels. In the hypertrophied tonsils they are much more numerous. Their significance is not entirely understood, but inasmuch as they are more or less characteristic of chronic inflammatory processes they probably indicate a chronic infection of the tonsillar crypts or the absorption of toxic substances therefrom.

In four of the 10 cases of endocarditis it was noted that pneumococcus-like organisms were found in large numbers or nearly pure in the tonsillar crypts, while they rarely occurred, as we have seen, in other conditions. This fact is interesting because these pneumococci are apparently identical with the common so-called endocarditic cocci. In one of the malignant endocarditis cases, as above stated, this organism was obtained in the tonsillar crypts nearly pure and in the heart's blood, pleural and pericardial fluids. The data at hand are not sufficient to permit definite assertions, but it appears that these organisms which are normally found on the buccal mucosa and which have been called *Str. viridans* (Schottmüller), *Str. salivarius* (Gordon), and are known by many other names, are not often found in the crypts. I am inclined to believe that under certain conditions they may invade the crypts, especially if the tonsils have been previously diseased, as in cases of acute articular rheumatism, and it is possible that their virulence may be thus increased and that they may enter the circulation from the diseased tonsil and localize on the valves of the heart. It

is known that such cocci obtained from normal mouths may produce experimental endocarditis in rabbits.¹

In this connection may be mentioned the work of Rosenow² on endocarditis. He has isolated by blood culture in a series of cases an organism which he believed to be a modified pneumococcus, and by intravenous and intracardiac injection of large and repeated doses into animals he was able to produce endocarditis with many of these strains. This organism appears to be the same as the organism isolated from the tonsils of heart cases described above, and like them too it very rarely produces arthritis in animals. He is of the opinion that in two of his cases the infection probably came from the tonsils, though apparently tonsillar cultures were not made.

It is not intended in this paper to discuss in detail the therapeutic effect of removal of the tonsils. It is sufficient to state that the improvement in many cases in the various clinical groups following tonsillectomy has been marked, resulting often in complete cure. In some the trouble was arrested while in a few no effect was noted. Favorable results have been obtained by many others, as is commonly known. Perhaps the therapeutic results furnish the best argument we have so far in favor of the idea that the infected tonsils bear some relationship to arthritic, renal, cardiac, and other clinical conditions. The evidence in this respect seems amply sufficient in many cases to localize definitely the source of trouble in the tonsils. Furthermore, the occurrence in the tonsils, as was found repeatedly in our series, of a pure or nearly pure growth of hemolytic streptococci with no evidence whatever, so far as our present technical methods indicate, of any other significant germ, would seem to point to this organism as the etiological organism in these cases.

The mere fact that these various strains of streptococci from tonsils are capable of causing joint lesions in animals is perhaps in itself not of great significance. It has been shown by others, particularly by Cole, Harris, and Beattie, that streptococci from various sources will produce arthritis in animals. I have pro-

¹ *Jour. Path. and Bact.*, 1911, 15, p. 323.

² *Jour. Infect. Dis.*, 1909, 6, p. 245.

duced arthritis in rabbits with typical hemolytic streptococci isolated from normal tonsils of individuals who never had had anything more than the usual colds common to nearly everyone. It is of interest that the various strains from cardiac, renal, arthritic, and other cases, and even the strains from normal tonsils, are apparently identical not only in their cultural reactions, especially in sugars, but also in their pathogenic properties in animals. I think it should be pointed out that while streptococci may be the causative organisms in many of these cases, they do not appear to be specific for the various clinical conditions. The reason why in one case nephritis is produced and in another, for instance, an arthritis, probably lies in the varying local susceptibility of the tissues of the individual rather than in any peculiar specificity of the infecting organisms.

SUMMARY.

In a series of cases including chronic articular, renal, and cardiac affections, and chronic tonsillitis, the crypts of the extirpated tonsils generally revealed the hemolytic streptococcus as the predominating organism and in some cases in practically pure growth.

A few exceptions were noted, especially in endocarditis, in which a pneumococcus-like organism was predominant.

The hemolytic streptococci are virulent for rabbits and other animals, invariably localizing in or about the joints and producing multiple arthritis. It only occasionally localizes on the heart valves. The pneumococci rarely produce arthritis but frequently localize on the heart valves.

The multiple arthritis in animals may become chronic, lasting for months. The exudate is mucoid early, later becoming purulent; usually it contains streptococci though in a few instances it was sterile.

The streptococci isolated from the various clinical groups of cases are alike in their morphology, their reactions in media including sugars, and also in their pathogenicity for animals.

The bacterial flora of the surface of the tonsils as revealed by a study of these chronic cases is usually strikingly different from the flora of the crypts. They may occasionally be similar but usually

they are not. Subcultures as ordinarily made from the surface of the tonsils, especially in chronic infections, are quite unreliable for determining the crypt flora.

The crypts of enlarged tonsils in nearly all cases contain large numbers of virulent streptococci and these cases may therefore be considered as streptococcus carriers.

A large part of this work was done at the Memorial Institute for Infectious Diseases, Chicago, and most of the material was obtained from the service of Dr. Frank Billings at the Presbyterian Hospital. Material was also received from Dr. J. L. Miller, Dr. Joseph Capps, Dr. George Shambaugh, Dr. Dyas, Dr. G. W. Post, and several other physicians, to all of whom I wish here to acknowledge my indebtedness and express my thanks.

THE AMEBACIDAL ACTION OF EMETIN.*

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The much-lauded, and as often denied, efficacy of ipecacuanha in the treatment of amebic dysentery was investigated experimentally by Vedder.¹ Briefly, he found that certain preparations of ipecac exerted a marked lethal action on cultures of saprophytic amebae, paramecia, and balantidia. The efficacy seemed to depend on the emetin content, and this fact pointed to the greater excellence of the Brazil root. While several preparations killed amebae in dilutions of 1:10,000 to 1:50,000, a preparation of de-emetized ipecac failed to kill in 1:5,000 solution. Emetin alone killed the amebae, paramecium, and balantidium in 1:100,000 solution. Vedder also found that when 2 per cent of the fluid extract of ipecac was mixed with agar it exerted a marked inhibitive and germicidal action on *B. typhosus*, *B. paratyphosus*, *B. dysenteriae*, and *St. pyogenes aureus*. This is of importance when one considers the like rôle played by symbiotic bacteria in amebic lesions.

The writer repeated part of Vedder's experiments as in his preliminary note no mention was made of the possible influence of body temperature or the lack of symbiotic bacteria on the results obtained.

TECHNIC.

A 1:10,000 solution of emetin was prepared by adding 0.01 gm. of Merck's emetin to 100 c.c. of sterile double distilled water. The alkaloid was put into solution by adding three drops of $\frac{n}{1}$ HCl. This solution was found to be sterile. Further dilutions were made with aseptic precautions with sterile double distilled water.

The ameba used is apparently of the *limax* type and was isolated on Musgrave and Clegg's medium from tap water in

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¹*Mil. Surg.*, 1911, 29, p. 318.

Oakland, Cal., in 1909. The culture is composed of the descendants of a single ameba growing along with a small actively motile, non-chromogenic, non-spore-bearing bacillus which produces no distinctive changes in any of the ordinary culture media.

The cultures to be tested were grown for a varying number of days in flasks containing 5 per cent + 1 broth in tap water; autoclaved at 127° C. One c.c. of the ameba culture was pipetted into the bottom of small, sterile, cotton-plugged test-tubes with precaution to avoid contaminating the sides of the tubes, and an equal quantity of any given dilution of emetin was run in on top. After carefully mixing the contents the tubes were incubated at the temperatures and for the length of time detailed below. The contents were then poured into flasks containing 100-200 c.c. of sterile 5 per cent broth, prepared as above, to determine the viability of the amebae. These flasks were then kept at 18°-24° C.

Owing to the rather powerful germicidal action of emetin, control tubes were always made and these were poured into flasks of 5 per cent broth which had been inoculated the day before with a pure culture of the symbiotic bacterium. This culture had been replated several times and numerous examinations proved it to be free of amebae. That this precaution was necessary may be seen from the fact that when the ameba-bacteria mixture was first plated in + 1 agar, amebae wandered out of several well isolated colonies.

EXPERIMENTS.

The action of emetin on the symbiotic bacterium.—1. The bacillus remained alive for several days when a large loop of the densely clouded water of condensation from a + 1 agar culture was inoculated into 1 c.c. of double distilled water. Similar inoculations were made into 1 c.c. of emetin-distilled water dilutions and subcultures into + 1 broth were made immediately afterward and at greater intervals. All negative subcultures were watched for several days to rule out possible inhibition. In all cases there was no immediate germicidal action. The 1:10,000 dilution yielded sterile subcultures when these were made 1.25, 5, and 22.5 hours after inoculation and incubation at 36°-37° C.

2. Emetin-distilled water dilutions 1:10,000, 1:50,000, and 1:100,000 were further diluted with an equal quantity of sterile 5 per cent broth and inoculated and incubated as in the preceding test. Subcultures were made at 48- and 72-hour intervals. Here only the 1:20,000 dilution yielded sterile subcultures.

The action of emetin on the amebae growing with the symbiotic bacterium.—Series 1. The broth culture of amebae was four days old and rich in trophozoites. No cysts were seen in several loopfuls examined. The ameba-bacteria mixtures were not killed by the 1:20,000, 1:100,000, and 1:200,000 dilutions of emetin after one hour's exposure at 37° C. All six flasks examined two weeks later showed many trophozoites and cysts.

Series 2. This was performed as above with a 34-day culture of amebae containing a large number of cysts. Here too the subcultures all showed amebae after one hour's exposure at 39° C. to the same dilutions of emetin.

Series 3. This was performed as above with a 15-day culture of amebae rich in trophozoites. An occasional cyst was found on thorough examination. In this series none of the subculture flasks showed amebae when examined thoroughly on the seventh and eighteenth day after inoculation with the above dilution mixtures, which had been kept at 36°–38° C. for 23.5 hours. Pure cultures of the symbiotic bacterium grew in all six flasks.

Series 4. This was performed with a four-day culture rich in trophozoites and it was only after repeated examinations that a few cysts were found. The same dilution mixtures were allowed to remain in contact for 24 hours at 34°–35° C. Here the subculture flasks were examined on the 10th day and 18th day after inoculation. In the 1:20,000 and 1:100,000 flasks amebae were present in one but absent in the control. Amebae were present in both of the 1:200,000 flasks.

Series 5. A culture of amebae in which no cysts could be found on repeated examinations but rich in trophozoites was used. Emetin 1:20,000 killed the trophozoites in 24 hours, at 37° C. A control, with the sterile distilled water with which the emetin dilutions had been prepared, yielded amebae in the subculture flask.

SUMMARY AND CONCLUSIONS.

Emetin in 1:20,000, 1:100,000, and 1:200,000, dilutions killed the amebae in one of the five series of experiments (3) after 23.5 hours exposure, at 36°-38° C. None of these dilutions was amebacidal in an hour. It seems fair to presume that when amebacidal action was manifested the emetin acted upon the trophozoites alone and that failure to kill may be attributed to the presence of cysts. While emetin in 1:20,000 dilution was found to kill the symbiotic bacterium in 48 hours it did not exert such an action in 24 hours in the amebae-bacteria mixtures. Exposure to body temperature for 24 hours did not kill this saprophytic ameba.

NUMBERS AND TYPES OF BACTERIA CARRIED BY CITY FLIES.*

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During the past decade much has been published in regard to the supposed danger from house flies as the disseminators of pathogenic bacteria. More or less evidence has been presented to the effect that these insects may, at times, spread a long category of infections, including, to quote the list of Purdy¹ (see also Hewitt²), typhoid fever, infantile diarrhea, bacillary dysentery, cholera, tuberculosis, diphtheria, erysipelas, contagious ophthalmia, cerebrospinal meningitis, anthrax, and possibly smallpox. This evidence has recently been summarized and discussed by Chapin³ in an interesting manner. For a number of these diseases the chain of evidence is incomplete, but for infections of fecal origin, the guilt of this ubiquitous insect has been clearly established in certain instances, especially as regards typhoid fever. Hamilton,⁴ Ficker,⁵ Klein,⁶ and Bertarelli⁷ have severally succeeded in isolating the typhoid bacillus from flies caught in the neighborhood of typhoid fever cases, although the attempts of other investigators under similar circumstances have been fruitless. Hamilton, first, in 1903, was able to prove that an outbreak of typhoid fever, restricted to a certain ward of Chicago where the street sewers were inadequate and the sanitary arrangements of most of the houses were of the worst, was in large measure due to flies acting as carriers of the specific bacilli, which, in fact, were isolated from a number of flies caught in two undrained privies, on the fences and house walls, and in the room of a typhoid patient. In 1910, Bertarelli, in an investigation of 100 flies caught in a household of the better class

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¹ *Jour. Roy. San. Inst.*, 1909, 30, p. 496.

² *Quart. Jour. M. Sc.*, 1909, 54, p. 347.

³ *The Sources and Modes of Infection*, New York, 1910.

⁴ *Jour. Am. M. Ass.*, 1903, 40, p. 576.

⁶ *Brit. Med. Jour.*, 1908, 11, p. 1150.

⁵ *Arch. f. Hyg.*, 1903, 46, p. 274.

⁷ *Centralbl. f. Bakt.*, I, Orig., 1910, 53, p. 486.

of Italians, who were suffering from an outbreak of typhoid, was successful in the isolation of the typhoid bacillus from the bodies of eight flies. Reed, Vaughan, and Shakespeare¹ have shown that flies were undoubtedly one of the most active agents in the spread of typhoid fever in the U.S. military camps during the Spanish War in 1898.

Although the guilt of the house fly has been clearly established in certain instances as a typhoid spreader, the relative importance of this vehicle of transmission as compared with the other well-known methods of transfer has been by no means clearly established. What is, perhaps, a timely warning of the danger, as regards the popular mind, of over-emphasis being laid on this mode of transmission has recently been voiced by Chapin. He believes that unwarranted faith in the iniquity of these insects may lead to the neglect of the far more serious danger of contact infection, and that a failure of a decrease in the death-rate from typhoid after an enthusiastic anti-fly campaign would tend to bring discredit on the well-grounded warnings of health officers. "It is probable," he concludes, "that under certain conditions, as in military and civil camps, and in filthy communities without sewerage, insects, especially flies, may be an important factor in the spread of fecal-borne diseases, but there is no evidence that in the average city the house fly is a factor of great moment in the dissemination of disease."

With the hope of gaining some additional light on the nature of the bacteria conveyed by flies which frequent a congested part of New York City, I have, during the past summer, investigated in detail the bacterial content of a considerable number of these insects which were caught entering the windows of the laboratory building. The south side of this building faces the rear of a row of tenements of the poorer grade with an intervening space of about 75 feet. The flies caught were among those which were continually circulating in and out of the open windows of these tenements. It seemed possible that the flies might have picked up bacilli of the dysentery type from the soiled diapers of infants sick with infective summer diarrhea, for without doubt such dejecta

¹ *Report on the Origin and Spread of Typhoid Fever in the U.S. Military Camps during the Spanish War, 1898.*

were occasionally exposed to them. Weather conditions, as it happened, were especially favorable for the work, as in the early part of the summer there was an extended period of exceptional heat and drought.

Methods.—The flies, as a rule, were examined in lots of 10. These were caught, without crushing, as they entered the open windows of the laboratory, and placed in a large sterile test tube. They were then shaken for five minutes in 10 c.c. of sterile normal salt solution, and the wash was set aside and labeled I. The flies were then rinsed thoroughly in 20 c.c. of the salt solution, which was drained off and discarded. The washed flies were next placed in 10 c.c. of the salt solution and the abdomens were so squeezed with a sterile platinum spatula that the content of the intestine exuded into the fluid. The thoroughly emulsified intestinal matter of the 10 flies was labeled II.

Platings were made from I and II, suitably diluted, in nutrient agar +1. to phenolphthalein, in litmus lactose agar, and on Conradi-Drigalski medium. These plates were incubated 24 hours at 37° C. The number of colonies on the nutrient agar plates was taken as the total count, although in one instance it was exceeded by the count on the litmus agar plate. The types of colonies which appeared to be dominant were isolated and identified, and in addition a special search was always made for colonies of the dysentery bacillus type. These several types were transplanted to the Hiss semi-solid tube medium and later subjected to cultural and agglutination tests.

In Table 1 are summarized the findings during the course of the summer. The mean temperatures were supplied by the local U.S. Weather Bureau, to whom grateful acknowledgement is made. In this table under I are given the total number of bacteria and the types of sanitary interest found on the surface of the flies; and under II the same data as regards the intestinal content.

The bacterial flora of flies examined in April and in early June included apparently few or no bacilli. It consisted, in fact, as Jackson¹ also found for that season of the year, of cocci commonly existing as saprophytes in nature and of a few molds. The counts in the few instances were low. That this homogeneous and non-fecal flora should persist to the end of spring is rather surprising. The high counts began to appear the last of June, and also an abrupt change in the character of the bacteria, the cocci giving place in large measure to the bacilli characteristic of fecal matter. At the end of the two weeks of excessive heat in July we find the record count, both as regards the number of bacteria on

¹ Report to Committee on Pollution . . . of Merchants Association of New York, 1907.

TABLE 1

Under I are given the total counts and the types of bacteria present on the surface of the flies, and under II the same data for the intestinal content.

DATE	MEAN TEMPERATURE	I			II		
		Total Count	Lactose Fermenters	Types	Total Count	Lactose Fermenters	Types
April 5...	48	330	50 all cocci	Albococcus Aurococcus	4,200		All cocci
June 5...	60	530	220 all cocci	Micrococcus flavus Albococcus pyogenes	1,800	800 all cocci	Micrococcus flavus No bacilli
" 9...	72	190	20 cocci		10	80	Mostly cocci B. coli communis
" 28...	78	17,000		Streptococcus equinus B. coli communis	42,000		
" 30...	71	6,600	300	Streptococcus fecalis B. coli communior	1,100,000	17,000	Streptococcus fecalis Streptococcus salivarius B. aerogenes
July 7...	79	59,000	1,000	B. coli communis B. acidi lactici	90,000	22,000	B. acidi lactici
" 10...	86	5,400	1,400	B. coli communis B. coli communior	97,500	24,000	Streptococcus fecalis B. coli communior B. acidi lactici
" 13...	78	4,400,000	1,000	B. coli communis	28,500,000	8,000	B. aerogenes
" 25...	70	600,000	1,000	B. lacti acidi	7,500,000		B. PARATYPHUS A B. coli communis
" 26...	70	1,400	10	Albococcus pyogenes	16,000	900	Streptococcus fecalis B. aerogenes B. fecalis alkali- genes
" 27...	71	570	10	Streptococcus equinus B. lacti acidi	280,000	200	Streptococcus salivarius B. coli communis B. aerogenes
Aug. 2...	74	17,500	12,000	Streptococcus equinus B. aerogenes B. paracoli Many proteolytic	3,850,000		B. fecalis alkali- genes B. paracoli Many proteolytic
" 10...	78	118,000	50,000	B. aerogenes B. paracoli	1,380,000	470,000	B. coli communis B. aerogenes B. acidi lactici B. paracoli
" 14...	76	1,600	0		2,700,000	1,700,000	Streptococcus fecalis B. aerogenes B. paracoli
" 17...	80	1,000	0		300,000	60,000	B. coli communis B. aerogenes B. paracoli
" 21...	71	810	10	B. xerosis Albococcus pyogenes	38,000	0	
" 28...	76	84,000	1,000	B. lacti acidi B. paracoli	125,000	4,500	B. coli communior B. paracoli

the surface of the flies and also within the intestines. The higher temperatures not only stimulate the flies to greater activity but also probably cause an increased bacterial development in the organic matter which they frequent. Irrespective of temperature, however, it was found throughout the summer that periods of richness of bacterial contamination would alternate with periods in which the flies were comparatively free from such pollution; and as a rule these lower counts on the surface of the insects were paralleled by a similar decrease for the intestinal content. Although the number of examinations were too few for a definite conclusion, it would seem that these fluctuations were due to the advent at regular intervals of swarms of newly hatched flies which had not an opportunity to infest the filth which constitutes the favorite pabulum of these household pests.

The figures of the table show that the surface contamination of these "wild" flies may vary from 570 to 4,400,000 bacteria per insect, and the intestinal bacterial content from 16,000 to 28,000,000. Taken as a whole the relation of the surface to the intestinal count was at the ratio of 1 to 8.6. It is probable that there is little or no increase in the number of fecal bacteria within the intestine of the fly and that no distinctive flora is developed as in warm-blooded animals. Accordingly the number of bacteria found constitute practically the sum total taken up with the insect's food.

Fecal bacteria of the colon group began to appear in notable numbers the last of June and at times were by far the dominant types, whereas at other times they entirely dropped out. An average of 10 days in which the counts were complete, excluding the excessively high count of July 13, showed that for the surface of the fly these colon group bacteria constituted 13.1 per cent of the total, with limits of none to 63 per cent. In the intestine the average was 37.5 per cent, with limits of none to 68 per cent of total count. This last average percentage of colon bacilli corresponds quite closely with the 39 per cent which Esten and Mason,¹ in one of the very few investigations bearing on this point, found to obtain for a large number of flies caught in the neighborhood of

¹ *Bull. 15, Agric. Exper. Station at Storrs, Conn., 1908.*

swill-barrels, stables, and within houses.¹ Of 39 cultures of lactose fermenters isolated and identified, 31, or 79·5 per cent, belonged within the colon-aerogenes group, and eight, or 20·5 per cent, were *B. acidi lactici*. In detail these 39 cultures were distributed as follows: *B. coli communis*, 10; *B. coli communior*, four; *B. aerogenes*, 17; *B. lactici*, eight. Esten and Mason also found that in their series the colon-aerogenes types predominated over the more innocent lactici-acidi type.

Of the 15 cultures of streptococci isolated, none was *Str. pyogenes*. To a certain extent the favorite breeding place of flies, viz., horse manure, is revealed by the presence of *Str. equinus* on the surface of the flies, and their selected food by the *fecalis* and *salicarius* forms especially characteristic of human dejecta and frequently encountered in the intestinal content of the insects. One streptococcus culture split inulin but in no other respect resembled the pneumococcus.

The most noteworthy finding was that of three cultures of *B. paratyphus*, Type A, on plates made from the intestinal contents of flies caught on July 25. It is quite possible that more cultures of this bacillus might have been isolated from these plates if they had not been discarded before the nature of the bacilli was realized. At the time the cultures did not grow typically in the glucose-gelatin agar tube medium, showing neither motility nor gas formation even when the medium was stirred, and in these respects simulating the dysentery bacillus. Subsequent tests, however, showed that they were typical paratyphoid bacilli in that they produced gas in dextrose, galactose, levulose, maltose, and mannite broth, acid but no gas in dextrin, and neither acid nor gas in lactose and saccharose media. Gelatin was not liquefied. They formed no indol in Dunham's peptone medium and gave rise to a typical fluorescence in neutral red glucose agar. In litmus milk they produced

¹ Since this paper was sent to press my attention has been called to a recent article by William Nicoll (*Jour. Hyg.*, 1911, 11, p. 381). The writer has investigated the types of bacteria occurring naturally both within the intestine and on the surface of house flies caught in dwelling rooms and outhouses. He isolated 27 different varieties of the colon bacillus. He found, in agreement with my results, that the colon bacilli of the communis and communior types occurred far more frequently than *B. acidi lactici*. He recovered *B. paratyphus*, Type B, from two flies, apparently infected outside the laboratory. He considers it more than likely that a considerable proportion of the bacteria in the intestine of the fly are simply passing through and never become resident, the colon bacilli tending to disappear and to be replaced by other organisms, frequently non-lactose-fermenting.

a slight acidity which continued for over two weeks and then gradually returned to the neutral point in two cultures and to a rather marked alkalinity in the third. That they belonged to the Type A group was shown by the following agglutination tests.¹

TABLE 2.
AGGLUTINATION OF FLY PARATYPHOID CULTURES WITH ANTI-PARATYPHOID SERUMS.

PARATYPHOID SERUMS	CULTURES					
	"Fly" 50	"Fly" 53	"Fly" 56	116	A	B
1. Anti-116.	5,000	300	300	5,000	2,000	50—
2. Anti-A (101)	500	100	100	200	8,000	50—
3. Anti-B (22)	50+	50—	50—	50—	500	16,000

Absorption experiments, which are the true tests of specificity, revealed the interesting fact that although one of these cultures agglutinated to the limit with the anti-A serum yet it absorbed none of the specific agglutinin for the culture A. On the other hand these cultures absorbed completely the specific agglutinin for culture 116 in its antiserum. In these absorptions the serums diluted 1-10 were treated in each instance with the 24-hour growth from four agar cultures. This was sufficient in each instance to exhaust almost or quite completely the agglutinin for the culture used in the absorption.

TABLE 3.
ABSORPTION OF ANTI-116 PARATYPHOID SERUM.

CULTURES	WITH FLY CULTURE 50		WITH FLY CULTURE 56	
	Before	After	Before	After
Fly 50	5,000	20+	5,000	500
Paratyphoid A.	2,000	500	2,000	1,000
Paratyphoid 116.	5,000	20+	5,000	20—
Fly 56	300	20

ABSORPTION OF ANTI-A PARATYPHOID SERUM.

CULTURES	WITH FLY CULTURE 50		WITH FLY CULTURE 56	
	Before	After	Before	After
Fly 50	500	20—	500	20—
Paratyphoid A.	8,000	8,000	8,000	8,000
Fly 56	100	20—

¹For the paratyphoid cultures A and B, I am indebted to the Department of Public Health of the American Museum of Natural History. These cultures reacted typically in litmus milk. The culture 116 was isolated in 1905 by Dr. Buxton from the blood of a typhoidal case in Bellevue Hospital. The antisera were produced with rabbits.

Inoculations into guinea-pigs of these fly paratyphoid cultures disclosed approximately the same degree of toxicity as the stock paratyphoid cultures. Feeding experiments with white mice resulted negatively.

The similarity of these paratyphoid cultures isolated from flies to our stock culture 116 raises the question whether the insects picked up the bacilli inside or outside the laboratory. No absolute proof on one side or the other can be offered, but the probabilities are very strongly against the flies having gained access to this culture, as more than the ordinary precautions were taken against such a contingency. The stock cultures are kept habitually in the ice-box and the rabbits were inoculated with emulsions of the bacilli killed by heating. It would seem then that beyond a reasonable doubt these paratyphoid bacilli were introduced into the laboratory by the flies from some outside source.

Since the discovery of the group of bacteria intermediate between the colon and the typhoid forms, a great deal of investigation has been devoted to the occurrence and distribution of these types. It has been found that members of the Paratyphoid B or hog cholera group may be the causative agents in a wide range of animal diseases, among which are mouse typhoid, cat and rat enteritis, parrot and sparrow enteritis, swine pest, calf diarrhea, mastitis and enteritis of cows. In man, aside from their elimination by individuals clinically sick with paratyphoid fever, Prigge and Sachs-Muke¹ found in an examination of the dejecta of over 5,000 persons, 70 apparently healthy eliminators of paratyphoid bacilli. Further, they determined that these healthy carriers may be divided into two sharp groups. First, individuals who might have been in association with cases or might have become carriers through sickness. In one case a woman, who was an eliminator of paratyphoid bacilli during one and one-half years, finally developed severe clinical symptoms of paratyphoid infection. In the second group were placed the individuals never in contact with paratyphoid infection. This group included 14 women, 16 men, and 25 children. Among these the paratyphoid bacillus was found only once in repeated examinations, doubtless indicating that the bacilli were

¹ *Klin. Jahrl.*, 1910, 22, p. 237.

ingested with food and at once eliminated, the time coinciding with the examination. In this second group also belong the positive findings of Rimpau¹ in three of 50 school children, and the positive results of Mayer² in a similar investigation of the dejecta of sound children. Mayer states that the paratyphoid bacillus was found viable in dried feces after one and one-half years. Seifert,³ Dorset,⁴ Uhlenhuth,⁵ and others have found the hog cholera bacillus in the intestines of sound swine. These several references to the literature have been made to emphasize the fact that members of this group of bacilli intermediate between the colon and the typhoid bacillus are by no means confined to the clinically sick; accordingly there is the greater probability that dejecta or other matter containing them may at times be exposed to flies.

The group of paratyphoid bacilli known as Type A, to which agglutination experiments indicate the fly cultures belong, occurs less frequently in nature than Type B. Aside from its presence in typhoidal cases and enteritis, Uhlenhuth reports its isolation from a normal young pig and Schone⁶ from sausage meat which apparently had given rise to an acute attack of vomiting and diarrhea. Ruediger⁷ isolated a paratyphoid-like bacillus from the heart blood of a dog whose brain was examined for Negri bodies. This bacillus, though culturally like paratyphoid, agglutinated alone in a serum immune to Type A and with that at no higher dilution than 1-200. Apparently the present instance is the first in which paratyphoid bacilli have been isolated from flies.

It is noteworthy that the three cultures isolated are not identical as regards their agglutinability nor in their action on litmus milk. It is not to be inferred from this fact that they represent different strains of this micro-organism, because, as will be indicated elsewhere, they fall within the limits of possible variants in certain paratyphoid cultures. It is probable that they were simultaneously picked up from the same material. What this material may have been can only be surmised, but it seems probable that it was the dejecta either of a paratyphoid case or more likely that of a chronic

¹ *Deutsche med. Wchnschr.*, 1908, 34, p. 1045.

² *Centralbl. f. Bakt.*, I, Orig., 1910, 53, p. 234.

³ *Ztschr. f. Hyg.*, 1909, 63, p. 273.

⁴ *U.S. Dept. Agriculture, Bull.* 2, 1905.

⁵ *Arb. a.d.k. Gsndtsamte.*, 1908, 27, p. 425.

⁶ *Ztschr. f. Hyg.*, 1910, 65, p. 1.

⁷ *Jour. Infect. Dis.*, 1911, 8, p. 486.

carrier of this bacillus. That the culture is pathogenic for man is indicated by its high degree of agglutinability in the serum immune to a strain isolated from the blood of a typhoidal case.

It would seem reasonable that not alone the bacilli on the surface of the flies should be taken into consideration in passing judgment on these insects as disease-spreaders, but also those within the intestinal tract, as flies are continually depositing their excreta on dishes used for food and on food itself.¹

The bacilli designated *B. paracoli* in Table 1 are also deserving of special comment. Certain of these cultures when first isolated were strikingly like the dysentery bacillus except that they produced a little more luxuriant growth on glycerin agar than the dysentery form. They showed no visible gas in Hiss semi-solid tube medium even when vigorously stirred and caused no outgrowth into the medium after an incubation of several weeks. They split the monosaccharids alone with the formation of acid, but not mannite, maltose, lactose, dextrin, or saccharose. In litmus milk there occurred a primary acidity with a bluish-green cream which gave way in four or five days to a final moderate alkalinity. They would not agglutinate, however, in serums immune to the Shiga and Y types of the dysentery bacillus and, except for one culture with which there was a positive reaction at 1-200, reacted negatively with the Flexner type serum. Subsequent tests, however, showed that these cultures produce a moderate amount of gas with dextrose and galactose broth in fermentation tubes. Apparently the Hiss semi-solid tube medium cannot be depended upon to reveal the gas-producing properties of certain non-motile bacilli. The greater number of the isolations of these bacilli, however, produced after three days incubation a clouding of the tube medium of varying degree, either in the form of a diffuse cloud or an outgrowth from the stab, but without gas formation. In litmus milk the primary acidity gave way to a final intense blue-black alkalinity. None of these cultures liquefied gelatin. Duval and Schorer²

¹ According to the findings of G. S. Graham-Smith (*Rep. to the Loc. Gov. Bd. on Public Health and Med. Subjects*, 1910, N.S., 40, p. 1) bacteria on the surface of the fly quickly die, whereas pathogenic bacteria which have been taken into the digestive tract may continue there in a viable condition for some time and gradually be eliminated. For this reason he considers the intestinal flora of more importance from a sanitary standpoint than that on the surface of the insect.

² *Studies from the Rockefeller Institute*, 1904, 2, p. 42.

in their report on the bacteriology of the summer diarrhea of infants described this bacillus as occurring quite frequently in the stools, and comment on its somewhat superficial resemblance to the dysentery bacillus. They apparently did not ascertain that these bacilli will form gas in dextrose broth and that they one and all produce indol in peptone water and also a yellowish or greenish fluorescence with gas in neutral red dextrose agar. Evidently this bacillus has not been recognized heretofore as belonging in the group of paracolon bacilli.¹ They comprise a fluctuating family in which the visible gas-producing property is frequently suppressed. For guinea-pigs they exhibit a pathogenicity fully equal to that of *B. coli communis* or *B. paratyphosus*.

A culture of a diphtheroid bacillus which was isolated from flies the last of August is also of interest, in that the bacilli were morphologically identical with certain granular forms of the Klebs-Loeffler bacillus. These bacilli when stained with Neisser's method exhibit blue-black dots, often occurring at each end of the bacillus, which were typically barred and not uniform in length. Here, however, the resemblance to true diphtheria bacilli ended, as this culture produced a thick staphylococcus-like growth on glycerin agar and elaborated no toxin in alkaline sugar-free broth. They resembled *B. xerosis* in splitting saccharose with acid production but not dextrin; accordingly they have been so designated.

SUMMARY.

1. Flies examined up to the latter part of June were free from fecal bacteria and carried a homogeneous flora of coccal forms.
2. During July and August there occurred periods in which the flies examined carried several millions of bacteria, alternating with periods in which the number of bacteria were reduced to hundreds. The scanty flora probably indicated the advent of swarms of recently hatched flies.
3. Fecal bacteria of the colon type were first encountered in abundance the early part of July.

¹ Probably certain, at least, of these bacilli are identical with that described by H. deR. Morgan and J. C. G. Ledingham (*Proc. Roy. Soc.*, 1909, Med. II, Part II, p. 133) and named by them "Morgan's bacillus." They consider this bacillus an important factor in the summer diarrheas of children, chiefly because of its occurrence in 63 per cent of selected cases. They were able to isolate this bacillus from flies caught in both infected and uninfected houses.

4. The bacteria in the intestines of the flies were 8.6 times as numerous as on the surface of the insects.

5. On the surface of the flies the colon group bacteria constituted 13.1 per cent of the total; and within the intestine 37.5 per cent of the total.

6. Of the lactose fermenters, isolated and identified, 79.5 per cent belonged in the colon-aerogenes group and 20.5 per cent in the acidi lactici group.

7. Fifteen cultures of streptococci, isolated and identified, were distributed among the equinus, fecalis, and salivarius groups. There were none of the pyogenes type.

8. The most important isolations were three cultures of *B. paratyphosus*, Type A.

9. Bacteria of the paracolon type causing a final intense alkaline reaction in litmus milk and fermenting only certain monosaccharids were frequently encountered during August.

THE COMPLEMENT FIXATION REACTION IN THE DIAGNOSIS OF CONTAGIOUS ABORTION OF CATTLE.*

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The disease commonly known as contagious abortion of cattle has been known to veterinarians and stock breeders for nearly a century. It was during the early part of the nineteenth century that the disease was first noticed in England and France; but it was not until about the year 1850 that it was recognized as a contagious disease.

In the year 1878 Lehnert made a study of the disease, at that time very prevalent in the European countries devoted to stock breeding. He was the first to call attention to the fact that a healthy heifer would abort after having received a vaginal injection of uterine exudate from an animal suffering from contagious abortion.

Brauer likewise succeeded in terminating pregnancy prematurely in cows, by inserting into the vagina placental fragments taken from animals which had recently aborted.

By means of these experiments the transmissibility of the disease was established. Its true pathology was not understood at this period, however, as it was generally supposed that it was a constitutional disease rather than a local one.

The work of Nocard, although unfruitful in its effort to isolate the microorganism responsible for the widespread disease, threw valuable light on its pathological nature. He concluded from his studies that contagious abortion was not a disease which affected the general health of the animal materially, but that it was confined exclusively to the uterine mucosa, the fetus and fetal membranes. Pathologists of today are able to add but little to the teachings of this venerable scientist.

It was in the year 1896 that Bang and Stribolt took up the problem which had now apparently rested for a period of nearly 10 years. These authors were the first to isolate the microorganism which is now considered as the specific contagium of abortion in cattle.

Having procured an animal which presented typical clinical symptoms of threatening abortion, they made preparations for the elaborate study which resulted in the discovery of the long-sought-for microbe.

The technic followed by Bang and Stribolt was the following:

The animal which they had chosen for their study was killed. The gravid uterus was then carefully enucleated and transferred to the laboratory under aseptic precautions.

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Tubes containing gelatin serum agar were inoculated from the amniotic fluid according to the ordinary method of anaerobic culture. The inoculated tubes were then placed at a temperature of 37°. After a period of from two to four days they noticed small colonies almost imperceptible to the naked eye developing just beneath the surface of the medium; this zone of growth varied from 1-2 cm. in depth. These colonies they described as being round, very small, some not larger than a needle point, others as large as a pin head. Examined under the microscope the edges of the colonies appeared serrated.

The microorganism thus isolated was found to be a cocco-bacillus measuring from 1-2 μ in length. It is non-spore-bearing, stains readily with the ordinary anilin dyes, and is gram negative.

Upon studying the biological properties of this cocco-bacillus further, Bang and Stribolt were impressed by its peculiar behavior toward oxygen. In view of the fact that the colonies developed just beneath the surface of the culture medium, the bacillus was designated as a semi-anaerobe, requiring for its development an environment in which the oxygen pressure was less than that of the ordinary atmosphere. This characteristic was found to be peculiar to the first generation only; the succeeding generations, although able to grow in ordinary atmosphere, were found to thrive best in an atmosphere of nearly pure oxygen.

Successful in their attempts to precipitate abortion in cows, sheep, and goats, with either intravenous or intravaginal injections of a pure culture of the cocco-bacillus, Bang and Stribolt concluded that this was the specific etiological agent of contagious abortion.

Following the technic recommended by their Danish contemporaries, McFadyean and Stockman of Great Britain, and Nowak of Austria soon succeeded in isolating an organism identical with that described by Bang and Stribolt.

McFadyean and Stockman also developed a method for the isolation of the cocco-bacillus of Bang which, in their hands, gave better results than the one above described. This consisted in growing a culture of *B. subtilis* in a closed chamber together with plates inoculated with material containing *B. abortus*. The culture of *B. subtilis*, in absorbing a part of the oxygen contained in the chamber, created an atmosphere favorable to the development of *B. abortus*.

Strange as it appears, American bacteriologists hitherto have not been as successful in isolating this organism as their European colleagues, though recently McNeal and Kerr have isolated a microorganism which they believe to be identical with the bacillus of Bang.

In view of this fact the opinion has been gaining credence in America that the specific agent responsible for the epizootic abortion in this country is not identical with that of Europe. The question of identity, therefore, was the first to arrive in the conduct of the investigation here recorded. It seemed probable that it could be solved by the complement fixation reaction, using a Copenhagen culture of *B. abortus* as antigen. The kindness of

Dr. Holth of the Royal Veterinary Laboratory of Copenhagen supplied the culture and this work was made possible. I wish to express my appreciation of the assistance given me by Dr. Holth.

The antigen consisted of a 10-day serum broth culture which was prepared as follows: 200 c.c. of ordinary broth, to which was added 50 c.c. of naturally sterile horse serum, were placed in a sterile Erlenmeyer flask of 750 c.c. capacity. The flask was closed by means of a rubber stopper perforated by two glass tubes, A and B, well plugged with cotton. Tube A extended deep into the culture medium to within 1 cm. of the bottom of the flask. Tube B merely perforated the stopper.

The flask was then placed at a temperature of 37° C. for 24 hours. If at the end of this time no bacterial growth developed it was inoculated with *B. abortus* and immediately oxygenated. The oxygen was gently led through a cotton filter into tube A for a period of about five minutes. The tubes A and B were then carefully sealed with paraffin, and the flask placed at a temperature of 37° C. for a period of 10 days. The antigen was then ready for use. The antigen thus prepared, if carbolized, will keep for months.

Hemolysin was prepared by injecting a rabbit, intraperitoneally, with 7 c.c. of a 1 per cent suspension of washed horse corpuscles, at intervals of five days.

On the 10th day following the fifth injection, the hemolytic titer of the serum was determined. It was found that 0.05 c.c. of a 1 per cent solution of serum was sufficient to hemolyze 0.5 c.c. of a 1 per cent suspension of washed horse corpuscles within 45 minutes.

The rabbit was then killed, and the blood, which was collected under aseptic precautions, defibrinated and centrifuged.

The serum, after being apportioned in hermetically sealed pipettes of about 0.5 c.c. each, was inactivated at 56° for half an hour and placed in the ice-chest to be kept for further use.

Guinea-pig serum served as complement. The titer of this complement and of the antigen was determined as indicated in the following tables:

Tubes	Guinea Pig Serum Dilution 1:4	Hemolysin 1 per cent	Red Corpuscles— Horse 1 per cent	NaCl Solution 9 gm. per Liter	Degree of Hemolysis
1	0.03	0.15	0.5	1.5	0
2	0.04	"	"	"	Partial
3	0.06	"	"	"	Complete
4	0.08	"	"	"	"
5	0.10	"	"	"	"

The titer of the complement in this case lies between 0.04 and 0.06 c.c. In the presence of antigen-serum of a normal animal (cow), 0.04-0.06 c.c. complement would not be sufficient to effect complete hemolysis. In practice it is therefore necessary to use more than this amount of complement.

The amount of complement necessary varies according to the nature and amount of antigen used. In the present investigation it has been found practical to use twice the titer of the complement, for which has been adopted the term "titer dose." In the instance here cited the titer dose would be 0.1 c.c. (2×0.05).

The following table illustrates the technic used in titration of antigen:

Tubes	Antigen	Complement (Dilution 1:4)	Hemolysin 1 per cent	Red Corpuscles— Horse 1 per cent	Inactivated Serum of Infected Animal (Cow)	NaCl Solution	Degree of Hemolysis
1	0.0	0.1	0.15	0.5	0.02	1.5	Complete
2	0.4	"	"	"	"	"	0
3	0.3	"	"	"	"	"	0
4	0.2	"	"	"	"	"	0
5	0.1	"	"	"	"	"	0
6	0.05	"	"	"	"	"	0
7	0.02	"	"	"	"	"	Partial
8	0.01	"	"	"	"	"	Complete

Thus it is seen that at least 0.05 c.c. antigen is required to inhibit hemolysis in the presence of the specific amboceptor (serum of infected cow).

In examining the sera of cattle to determine whether or not they are infected with contagious abortion, the procedure adopted was the following:

Tubes	Antigen	Complement (Dilution 1:4)	Hemolysin 1 per cent	Red Corpuscles 1 per cent	Suspect Serum Inactivated	NaCl Solution	Degree of Hemolysis
1	○	○	○	○.5	○	1.5	■
2	○	○.1	○.15	..	○.02	..	Complete
3	○.15	○
4	○.02	..	○
5	..	○	○
6	..	○.1	○
7	○.01	..	○
8	○.005	..	Partial

The above table represents a positive reaction.

In practicing the complement deviation reaction it is necessary to add to the tubes containing the specified amount of NaCl solution, the antigen, the suspect serum, and the complement; this being done the tubes are placed in the thermostat at 37° for one hour in order to allow the complement time to become fixed. At the end of the hour the hemolysin and red corpuscles are added, and the tubes again placed in the thermostat and left for two hours. At the end of this time the reaction is noted.

Up to the present time there has been occasion to examine for the purposes of this study the sera of 76 animals. In the following table is submitted a brief history of each animal and the result of the examination:

History	Number	Reaction
Never aborted.....	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 47	—
Infected herd:		
Never aborted.....	51, 52, 53, 67, 69, 70	—
" (two-year-old)	50	+
" (three-year-old; calved once)	49	+
" calved.....	41 (two-year-old)	+
" (two-year-olds)	43, 45, 46	+
Aborted one year ago.....	54, 56	+
" (two-year-olds)	55	—
Aged, aborted years ago.....	44	—
Aborted three times in succession.	57	+
Carried calf full period	15 (calf died 15 minutes after birth)	+
One-year-old.....	68	—
Aborted 1910.....	71, 72, 73, 74, 75, 76, 63, 65	+
" (now 5 mos. pregnant)	64	+
Aborted 1911.....	4 (January 30), 42 (May 20)	—
" (March 10), 18 (January 2), 19 (February 19),	17	+
" (March 10), 40 (August 1), 39 (February 19)	38	+
Aborted 1910 and August 2, 1911...	66	+
Aborted last two years.....	62, 60 (now 5 mos. pregnant)	+
Aborted.....	58 (last year), 59 (3 years ago)	+
" one year ago.....	48	+
" three years ago.....	61 (since carried 2 calves to term)	+
Calf 4½ mos. old, born prematurely.	21 (dam reacts)	—

The results shown in the table are to be summarized as follows:

Of the 76 animals examined, 32 gave a positive reaction and 44 a negative. Of the 32 which gave a positive reaction, 25 have aborted. Six animals which reacted have never aborted. These were all without exception in infected herds. One animal from an infected herd which reacted carried fetus full term, but calf died 15 minutes after birth.

Of the 44 animals which reacted negatively, two have aborted. These two abortions may have been due to other causes. One of these two animals was in an infected herd, the other not. Of the 44 animals which gave a negative reaction, 10 were in infected herds.

While this work was being carried on attempts were made to isolate the etiological agent of contagious abortion in this country. Thus far seven fetuses from different sections of the state have been examined.

The procedure was as follows:

Tubes of liquefied 1 per cent agar were placed in a water bath at 55° for 15 minutes. After this time there was added to the agar one-fifth its volume of naturally sterile horse serum at the same temperature. The tubes of serum-agar thus formed were then cooled to 42°, and inoculations were made from the gastro-intestinal tract of the fetus. The inoculated tubes were immediately placed in cold water, to insure a rapid solidification of the culture medium.

Tubes of slant serum agar were likewise inoculated from the gastro-intestinal tract of the fetus, and placed in a closed chamber together with cultures of *B. subtilis*. This is a modification of the method recommended by McFadyean and Stockman.

Both of these procedures were successful in cultivating a cocco-bacillus which corresponds in detail, culturally and morphologically, to the cocco-bacillus first described by Bang and Stribolt. The organism was isolated from five of the seven fetuses examined.

It was further found in this investigation that this cocco-bacillus would grow very well on the surface of slant serum agar provided care was exercised to seal the tubes hermetically immediately after inoculation. The culture medium, which had previously been

deprived of oxygen by being heated, undoubtedly absorbs a sufficient amount of the oxygen contained in the tube to create an atmosphere favorable to the development of the bacillus abortus.

It has been said that the organism in question will usually be found in pure culture in the gastro-intestinal tract of the fetus. In this study contamination was found to be the rule.

In order further to establish the identity of the cocco-bacillus which had thus been successfully isolated, resort was had once more to the complement fixation reaction, this time, however, the cocco-bacillus isolated as described above being used as antigen.

For this work 15 animals were chosen, the sera of which had previously been examined, using the Copenhagen culture as antigen. Nine of these animals had given a positive reaction, and six a negative.

The reactions in these two series coincided in every instance. These experiments then, it may be believed, have proved beyond question that the European disease and the American disease are identical.

CONCLUSIONS.

1. Contagious abortion of cattle in this country is caused by a microorganism identical with that causing the disease on the European Continent.

2. The complement fixation reaction is a reliable and accurate method of diagnosis.

3. All animals do not contract the disease, even if in an infected herd and living under the same conditions as those which become infected.

4. An animal may react positively, indicating that she has at some period been infected, and yet may not abort. This brings up the question of immunity, which will be the subject of a future study.

I wish to take this opportunity of expressing my thanks to Dr. F. B. Hadley for the assistance he has rendered me.

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STUDIES ON PELLAGRA BASED ON ITS OCCURRENCE
IN 1910 IN THE COOK COUNTY INSTITUTIONS AT
DUNNING, ILLINOIS.*

F. B. CLARKE, RALPH C. HAMILL, L. J. POLLOCK,
ARTHUR H. CURTIS, AND GEORGE F. DICK.

CLINICAL MANIFESTATIONS.

F. B. CLARKE.

Pellagra has been recognized for almost 200 years as occupying an important place among the various diseases common to the countries in southern Europe, but it has been only within the past few years that it has been recognized in this country. In all probability the disease has been prevalent in various states, but it was not until the epidemic at the Alabama State Hospital in 1906, reported by Searcy in 1907, and the epidemic in the South Carolina State Hospital at Columbus in 1907 that the interest of the medical profession was aroused to the true situation. The activity of Babcock in his careful study of this epidemic combined with the interest taken by the State Board of Health resulted in bringing about the National Conference on pellagra at Columbia, S.C., November, 1909. This conference has been quite potent in stimulating not alone the medical profession but various state boards of health to active interest in the study of this disease which has always been considered peculiar to certain other countries and which is seen to be of wider distribution and greater importance than at first assumed.

Gasper Casal of Spain in 1735 was the first to recognize and describe this disease as a clinical entity, although it is quite probable that it existed unrecognized for a number of years. It is easy to believe that its varied clinical manifestations were classified according to the peculiar symptomatology of each individual case, since many observers in this country now remember cases which were presumably pellagra, in which the findings were such as to lead to a diagnosis of gastro-intestinal disturbance, nervous or mental disease, or some unusual type of skin lesion.

Because of the peculiar rough condition of the exposed skin, the name pellagra (*pelle*, skin and *agra*, rough) was so constantly associated with the disease as to lead to this name being universally adopted. For years, however, the various countries

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preferred the names Alpine Scurvy, Psycho-neurosis Măldica, Astorian Leprosy, and many others, depending largely upon the country from which the disease was reported. In a few years the distribution was recognized as including practically all the countries of southern Europe. A large percentage of the population of the various provinces of the different countries was afflicted by this disease. Strambio in 1784 estimated that about one-twelfth of the population of Italy was pellagrous. In 1881 the number was considered to be about 104,000, but it has shown a decided decrease until at the present time it is estimated that there are perhaps 50,000 cases. In Spain Trilliers estimates that at the present time 20 per cent of the population of certain provinces is affected by this disease. The disease has not been confined to the countries of Europe alone, but epidemics have occurred in northern Africa and Mexico.

The report of a few isolated instances of this disease in the United States, such as the report of a case of pellagra in 1864 by John P. Gray of Utica, N.Y., and the report of a case by Bemis occurring at the Charitable Hospital, New Orleans, did not attract any special attention. It was not thought that pellagra would ever become epidemic in a country where the social and economic conditions were so much better than in the countries where the disease was so widely prevalent. In fact, writers of our most recent medical works did not consider the question of enough importance to devote more than a few paragraphs to it. Kerr called attention to the fact that pellagra was probably prevalent among the soldiers confined at Andersonville during the War; and it would seem quite in accordance with the prevalent conception of the etiology of the disease.

The first authentic report of an epidemic in the United States occurred at the Mount Vernon Alabama Asylum for negroes in 1906; and was reported by Searcy in 1907; within a few months the officers of the State Hospital for the Insane at Columbia, S.C., reported the occurrence of the disease in their institution. After a careful study of the disease in Italy, J. W. Babcock and J. J. Watson, in 1908, established the fact that pellagra as it existed in the United States was the same disease that existed in Italy. In November, 1909, a National Conference on pellagra was held in Columbia, S.C., under the auspices of the State Board of Health; this conference was attended by many from various parts of the country, and has been the means of stimulating interest in the study of the disease. At the time it was estimated that about 5,000 cases had been recognized in the past five years and that the disease was prevalent in about 13 states of the Union. The existence of pellagra in the southern states did not attract especial attention in Illinois, until Pollock in June, 1909, called attention to the existence of pellagra in the Cook County Institutions at Dunning. In August of the same year Zeller reported the existence of pellagra in the Peoria State Hospital, and by November 1 he had recognized 130 cases of the disease. At the present time pellagra has been observed in 33 states. One should not get the impression that it is essentially a disease of institutions. In Italy, where it is estimated that there are 50,000 cases each year, only 4 to 10 per cent are found to suffer from some form of mental disease. In Illinois, where about 250 cases have been reported, it is fair to assume that a much larger number of cases have been recognized; in the United States, however, statistics come largely from hospitals for the insane and as yet a definite ratio of the sane to insane pellagrins is not available.

The epidemic in the Cook County Institutions in 1910 involved 35 cases in all, 24 cases occurring in the insane, four developing

among inmates in the Infirmary, and seven in the Hospital for Tuberculosis.

The dietary of the institution is about the same as in other institutions of like character. During the year 1909, in which the first epidemic was recognized, various preparations of corn, such as corn meal mush, corn bread, and hominy, were served several times each week. Since then these various articles were eliminated out of respect to a belief that corn was the principal offender. Nevertheless the disease reappeared in 1910. There were recurrences in several cases who had pellagra the previous year, and the disease also appeared in a number of cases who had been inmates during the epidemic but had not been afflicted. The meal used was what was known as No. 2 and was not spoiled. While the results obtained by the elimination of corn products from the dietary of one year was not at all conclusive, yet it is in accordance with the results obtained in other institutions.

The cases occurred among patients of different nationalities, but none developed in negroes. The ages ranged from 11 to 62 years. The two sexes were affected equally.

The institution life of our cases ranged from three weeks to 31 years with the exception of one case, who was brought in with the disease in its last stages. It is rather interesting to find that in nine cases the residence was less than six months.

The dry type of skin lesion predominated throughout the epidemic, there being only two in which the lesions were of the wet type. The lesions usually appeared on the dorsum of the hands and fingers, rarely extending above the styloid process, as a bright red erythematous area, nearly always symmetrical, which soon became darker, with a thickening of the skin which in the greater number of cases after a few days became markedly fissured, followed by a decided desquamation which in many cases continued for a period of weeks. In nine cases the same type of lesion appeared upon the face, usually being limited to the forehead and malar prominences, and persisting about the same length of time as the lesions upon the hands.

The distribution of the lesions is not the same in both sexes, since among women the lesions appeared upon the face more frequently.

Only one patient, a woman, developed a lesion on the forehead without involvement of the skin of the hands, and in the two wet cases the lesions involved the face and hands in one, and the hands only in the other. There was no involvement of the skin of the unexposed surfaces except in two cases, in which there was a marked erythema of the labia majora of the skin of the perineum and inner surfaces of the thighs. In one of these two cases the tissues became quite edematous, later becoming excoriated and covered with a gray foul-smelling secretion; both ran a rapidly fatal course.

The intensity of the skin lesions was in direct proportion to the severity of the other symptoms, both of the wet cases proving fatal in a comparatively short time. It is interesting to note that during the epidemic of the preceding year the wet type of skin lesion predominated and the mortality was 67 per cent, as compared with 57 per cent this year. Gastro-intestinal symptoms were usually present before the skin lesions appeared, as shown by a failing appetite and loss of weight. In nine cases the skin lesions had been present on an average of three weeks before diarrhea developed; on the other extreme five cases showed well-marked diarrhea as long as three months before skin lesions appeared. In six cases showing marked nutritional disturbances there was no diarrhea.

In those cases with marked diarrhea there was stomatitis of varying grades; the mucous membranes of the mouth being markedly hyperemic, the tongue usually small with intensely red margins. Only a few of the rapidly fatal cases developed ulcers on the tongue and gums, which were accompanied in all cases by a marked increase of the flow of saliva.

The severity of the gastro-intestinal symptoms varied and was in direct relation to the fatality of the disease. The mental symptoms of the cases occurring among the insane usually showed a marked change regardless of the psychosis of the patient; most of the cases becoming apathetic and later stupid; the degree of stupor being in almost all instances in direct relation to the severity of the gastro-intestinal disturbances.

Neurological examinations early in the course of the disease did not show anything worthy of note; later the mental conditions

were such that careful observations could not be made, only a few of the cases had increase of the deep reflexes, and in three cases there were marked tremors and choreiform movements before death. In one case of Huntington's chorea the choreic movements were markedly increased.

The count of the red blood cells was comparatively high, considering the marked nutritional disturbances, and the hemoglobin was high in proportion to the number of red cells; the count of the white blood cells was in the greater number of cases high, and there was a relative lymphocytosis.

The course of the disease was quite varied, ranging from three weeks to five months, the average length of life after the diagnosis was made was six weeks, and in those cases which recovered the average length of time was about two and a half months.

Complicating diseases such as paresis, dysentery, and pulmonary tuberculosis were comparatively frequent, and the course of pellagra in these cases was quite rapid.

Treatment was symptomatic and empirical as regards medication; atoxyl and Fowler's solution were used but without benefit. The skin lesions were in some instances treated by excluding light and by application of ointments, with no apparent benefit. The diarrhea did not respond to medication of various kinds; three of the cases, complicated by Shiga bacillus infection, were improved by autogenous vaccine prepared by Dr. Dick; blood transfusions were performed in twelve cases by Dr. Pollock and Dr. Arthur Curtis.

EXAMINATION OF THE CENTRAL NERVOUS SYSTEM IN SEVEN CASES OF PELLAGRA.

RALPH C. HAMILL.

The only change that was at all common was a more or less well-marked chromatolysis in the anterior horn cells, in the cells of Clarke's column, and the large pyramidal and Betz cells of the cortex. This varied from merely an increased depth of staining of the entire cell to a complete loss of tigroid substance. In one case only did the cells appear perfectly normal, stained with Nissl.

In two cases the cells stained with toluidin blue, thionin, or cresyl violet, completely lost the stain in the differentiating alcohol. The tissue in one case had been fixed in Hatai fluid, in the other in 10 per cent formalin followed by Hatai. These cells stained with hematoxylin showed in one case, that fixed in Hatai, some apparently normal cells, but others with destruction of the tigroid and eccentric nuclei. In the other case the cells were uniformly shrunken and deeply staining.

In but two cases was there any manifest change in the white matter of the cord. In one, Marchi's method showed degeneration in the direct and crossed pyramidal tracts with a small amount of subpial degeneration in the dorsal columns, more in the upper dorsal than in the lower. In this case there was a round cell infiltration of the pia-arachnoid and surrounding the vessels, with some thickening of the walls, not of a syphilitic nature. In the other case, Weigert Pal stain showed well-marked degeneration in the posterior columns of the cervical, dorsal, lumbar, and upper sacral regions.

In two cases there was considerable hyperemia. In the others the vessels and meninges were apparently normal.

These changes agree with what has previously been found in pellagra. The only change in the central nervous system found frequently enough to be considered at all characteristic is a more or less marked degeneration of the tigroid substance of the anterior horn cells and of the large cells of the cortex.

TRANSFUSION OF BLOOD IN PELLAGRA.

L. J. POLLOCK AND ARTHUR H. CURTIS.

The form of pellagra among the patients of the Cook County Institutions was severe, the disease running its course essentially uninfluenced by the most approved therapeutic measures. Despite the utmost diligence in the treatment and nursing of these patients, the death-rate during the years 1909 and 1910 was 67 per cent and 57 per cent. The favorable report of Cole and Winthrop¹ therefore aroused especial interest in the possible value of trans-

¹ *Jour. Am. M. Ass.*, 1910, 54, p. 1354.

fusion for cases with grave prognosis. These writers report transfusion of 11 pellagrins, from the results of which they concluded that "transfusion is of undoubted value in certain severe and apparently hopeless cases." In their opinion blood obtained from a cured pellagrin possesses no advantages over that from a normal individual. They have since reported nine additional cases, making a total of 20, with eight deaths, 12 recoveries, and one relapse.

In view of Cole and Winthrop's encouraging report, which was quite in contrast with results from other forms of treatment, we decided to perform transfusion in a series of selected patients with decidedly grave outlook. In all except one instance our transfusions were performed by means of the Crile canula. One transfusion was performed according to the method described by Curtis and David.¹ Our series consists of 12 cases which may be divided into three groups, (1) moribund and dying; (2) very sick, practically hopeless cases; (3) those with active and severe pellagra. Six patients were of the first, four of the second, and two of the third groups. Patient 1 of group 1 was transfused with the blood of a cured pellagrin. All other donors were normal individuals. A total of 16 transfusions were performed, four pellagrins being twice transfused.

SYNOPSIS OF CASES.

Name	Group	Condition	Duration of Disease	Result
M.M.	1	Comatose	4 months	Death in 30 hours
S.R.	3	Fair	2 months	Recovery
M.O'C.	3	Poor	2 months	Recovery
N.S.	2	Rapidly failing	2½ weeks	Recovery
J.K.	2	Very weak, failing	2 months, 10 days	Recovery
L.G.	2	Critical	1½ months	Death in 15 days
A.L.	1	Comatose	2 months	Death in 6 hours
D.W.	1	Stuporous, dying	2 weeks	Death in 7 days
T.O.H.	1	Stuporous, dying	1 month	Death in 3 days
R.S.	1	Death impending	2 weeks	Death in 15 days
L.S.	1	Moribund	Unknown	Death in 12 hours
T.F.	2	Very weak, failing	1 month	Recovery

1. M.N. Admitted June 8, 1909. Diagnosis, constitutional inferiority. Female, age 35.

1/25/10. Developed diarrhea, pain in abdomen, incessant crying, depression, stomatitis.

3/25/10. Patient's symptoms increasing, diarrhea worse; rapidly failing.

¹ *Jour. Am. M. Ass.*, 1911, 57, p. 1453.

- 4/25/10. Skin now shows slight roughness. Patient has become completely comatose, heart action very weak, pulse threadly, slow respiration, death impending.
- 4/26/10. Transfused with blood of cured pellagrin
- 4/27/10. Died at 9:00 P.M.
2. M.O.C. Admitted October 21, 1909. Diagnosis, dementia praecox. Female, age 22.
- 5/15/10. Dermatitis of lower eyelids. Has diarrhea.
- 6/15/10. Lesions spreading; loss of weight.
- 7/11/10. Rapidly losing weight, has become very noisy, much weaker; quality of skin poor, muscles soft and flabby.
- 7/13/10. In a critical condition.
- 7/14/10. Transfused.
- 8/1/10. Fully recovered.
3. N.S. Admitted April 28, 1910. Diagnosis, dementia praecox. Female, age 24.
- 8/28/10. Developed pellagra with marked symmetrical dermatitis over wrists, hands, forehead, and face. Tongue red, marked diarrhea, rapidly losing weight, suffering from a severe intoxication.
- 9/8/10. Transfused.
- 9/30/10. Gaining rapidly in weight, skin lesions disappearing. Bowels normal.
4. L.G. Admitted February 27, 1908. Diagnosis, dementia praecox. Female, age 35.
- 2/27/08. Suffering with chronic pulmonary tuberculosis.
- 6/19/10. Has developed symmetrical dermatitis of both hands and forehead, losing weight; has diarrhea.
- 8/13/10. Acute exacerbation of above condition with marked weakness and unsteady gait. Condition critical.
- 8/20/10. Transfused; thrombus locally before desired amount transfused.
- 8/26/10. Transfused, successfully.
- 9/11/10. After a noted improvement patient has developed a new erythema over forehead and one mastoid. Dysentery marked. Patient is entering into a stupor and is developing bed sores.
- 9/30/10. Died.
5. S.R. Diagnosis, dementia praecox. Female, age 37.
- 5/1/10. Developed erythema over backs of both hands, giving way to desquamation. Beginning dysentery. Patient rapidly losing weight.
- 7/8/10. Transfused; thrombus formation locally during transfusion.
- 9/23/10. Transfused successfully, followed by marked improvement.
- 10/1/10. Recovered.
6. R.S. Admitted May 7, 1908. Diagnosis, mania-depressive. Female, age 50.
- 9/3/10. Has lost considerable weight. Shows marked weakness. Pigmentation over hands, roughening and fissuring of knuckles; is semi-stuporous. Death impending.
- 9/20/10. Transfused.
- 9/24/10. Tongue, red, beefy, marked stomatitis. No diarrhea. Patient stuporous.
- 10/5/10. Failed rapidly and died.

7. T.O.H. Diagnosis, cerebral syphilis. Male, age 34.
9/ 1/10. Patient has been failing physically for the past month. Has developed erythema over forehead, stomatitis, intermittent dysentery; failing rapidly.
9/17/10. Transfused. Thrombosis.
9/23/10. Patient weaker. Diarrhea increased. Extremities spastic, semi-comatose.
10/ 1/10. Transfused. Successful operation.
10/ 4/10. Rapidly failed and died.
8. A.L. Admitted August 15, 1907. Female, age 27.
6/ 1/10. Temperature 103° F. Marked leukocytosis. Temperature running septic curve. Probably acute endocarditis.
6/15/10. Recovered.
7/ 7/10. Continues losing strength. Apathetic, stuporous, grayish pigmentation of both hands.
8/ 7/10. Bright scarlet eruption over face. Tongue red. Dysentery. Patient failing.
8/25/10. Condition critical. Respirations 12 per minute. Pulse felt with difficulty. Deep coma. Transfused 11:00 A.M.
8/25/10. Died, 6:00 P.M.
9. J.K. Admitted June 18, 1908. Diagnosis, dementia praecox. Female, age 48.
8/ 8/10. Has lost weight. Developed period of excitement. Has symmetrical dermatitis of hands. Lips dry and cracked. Tongue red. Dysentery.
8/18/10. Transfused.
8/21/10. Patient rapidly improving. No diarrhea.
10/ 1/10. Recovered.
10. D.W. Admitted June 23, 1910. Diagnosis, dementia praecox. Male.
6/23/10. Pulmonary tuberculosis.
8/20/10. Has developed erythema over back of hands. Tongue red. Salivation, marked stomatitis. Commencing to fail rapidly. Symptoms more marked. Diarrhea increasing. Stuporous; has rapid pulse. Death imminent.
9/ 3/10. Transfused.
9/ 8/10. After temporary improvement redeveloped stupor.
9/10/10. Died.
11. L.S. Admitted October 14, 1910. No history obtainable. Patient admitted in state of collapse, semi-stuporous. Marked weakness. Vaginal mucous membrane and mucous membranes of mouth and conjunctiva very red. Has marked enteritis with discharge of fetid greenish stools. Tongue red, papillae prominent. Desquamation of skin over dorsal surface of both hands. Redness and fissures over knuckles. All deep reflexes lost.
10/16/10. Transfused. Patient's pulse stronger. Aroused from stupor.
10/17/10. Died at 2:00 A.M.
12. T.F. Admitted October 13, 1910. Diagnosis, dementia praecox, pulmonary tuberculosis.

- 9/25/10. Beginning to lose weight. Weakness, marked diarrhea, lunule not developed over the dorsum of hands, skin atrophic and red. Over the nose and face are similar lesions. Tongue and mucous membrane red.
- 10/19/10. Unsuccessful transfusion.
- 10/22/10. Successful transfusion.
- 11/7/10. Skin lesions have disappeared, patient has gained 20 pounds in weight.

A résumé of the results obtained reveals the following: Death eventually resulted in spite of transfusion in all six patients of group 1, in one of group 2, and in none of group 3. The mortality in these selected severe cases consequently did not fall much below the mortality from pellagra in general. The transfusion performed on those patients who were moribund was unavailing in our hands. The most that can be claimed is that the heart action was strengthened, the color and appearance of the skin improved, and the patient temporarily aroused from stupor, only soon to revert into the condition which existed prior to transfusion, with death the ultimate outcome. Of the four patients representing group 2, one showed temporary improvement followed later by recurrence of severe symptoms and death. Three made marked improvement with final recovery from the existing attack of pellagra. The patients of group 3 improved immediately after transfusion with resultant rapid disappearance of all signs of the disease.

CONCLUSIONS.

In our experience transfusion in pellagra is of value chiefly because it increases the general resistance of the patient. Transfused blood has a life of three weeks, at the end of which time the corpuscles undergo the natural laws of senescence. No special selective stimulating action was observed to result from the introduction of normal blood into the circulation of pellagrins. Such beneficial results as were undoubtedly obtained we would expect to result from the transfusion of patients afflicted with other wasting diseases. With the development of a simplified technic in the performance of which but little time is required and the amount of blood can be measured, we would recommend repeated transfusions in severe cases of pellagra. Under such conditions it may prove of value in the treatment of this dreaded disease.

INOCULATION OF MONKEYS WITH PELLAGROUS BLOOD AND
SERUM, AND THE OCCURRENCE OF B. MAYDIS
IN PELLAGRA.

GEORGE F. DICK.

(From the Memorial Institute for Infectious Diseases, Chicago.)

The investigation into the etiology of pellagra as it occurred in the Cook County Institutions was directed toward determining:

- (1) The infectivity of the blood of pellagrins for the monkey.
- (2) The toxicity of the blood of pellagrins for the monkey.
- (3) The bacteria associated with pellagra.

INFECTIVITY OF THE BLOOD OF PELLAGRINS FOR THE MONKEY.

Monkey 1.—Received subcutaneously 5 c.c. of defibrinated blood obtained the same day from a very severe case of pellagra which terminated fatally the following day. The monkey remained well as long as it was kept under observation (about three months). During the first month the temperature was taken twice daily and showed no change from the controls taken on the days previous to the injection.

Monkey 2.—The femoral vein was isolated, and 10 c.c. of blood, removed from the median basilic vein of a well-marked case of pellagra, were injected before clotting occurred. The wound was sutured and healed by primary intention. The monkey remained healthy.

THE TOXICITY OF THE SERUM OF PELLAGRINS FOR THE MONKEY.

Monkey 3.—Serum, obtained from a patient with pellagra preparatory to transfusion, was injected subcutaneously in 5 c.c. doses on alternate days. The serum was preserved in the ice box and kept sterile. The monkey received in all 60 c.c. of serum given in a period of 24 days. No change in temperature was observed, but the monkey became emaciated and died one week after the last injection. No symptoms, commonly considered characteristic of pellagra, developed. The autopsy showed an atrophy of the organs without marked degeneration. The brain and cord were somewhat softer than normal, and examination of

the cord showed marked chromatolysis of the ganglion cells of the anterior horns. Hemolysins and precipitins for normal human serum did not develop. Cultures from the heart's blood and peritoneal fluids were sterile.

Monkey 4.—Given 200 c.c. of serum obtained from a case of pellagra with well-marked skin lesions but in good general condition. The serum was given in 10 c.c. doses on alternate days. The monkey remained healthy.

Monkey 5. (Control).—Received injections as in the case of monkey 4, except that serum, obtained from a bleeding of a patient dying of uremia, was used. This monkey also remained healthy.

BACTERIA ASSOCIATED WITH PELLAGRA.

Bacteriologic examinations of the stools, skin lesions, mouth lesions, and blood were made. The blood in the few cases examined was sterile. The mouth and skin lesions yielded no special results, the common pus-forming cocci being the usual organisms present.

The stools of a number of cases of pellagra were examined as follows: The stools were taken to the laboratory in sterile test tubes and plated on litmus lactose agar as soon as possible. After 24 hours' incubation, subcultures of colonies, which did not resemble those of the colon bacillus, were made and the cultural characteristics tabulated. Control cultures were made from patients with diarrhea without pellagra.

In this way the bacterial flora of the stools of 20 cases of pellagra were compared with 25 cases of dysentery without pellagra.

Of the large number of bacteria isolated, only one organism was found to be present in more than one pellagrin and absent in the control cases. This organism was found in nine cases of pellagra. The organism is a motile, gram-positive bacillus resembling the anthrax bacillus in shape and size. It grows profusely on ordinary media with the formation of slimy opaque white colonies with a fairly sharp border. Gelatin stab cultures are liquefied in the shape of a cup at the top of the stab in about one week. Central spores are formed. The organism does not ferment dextrose, lactose, nor mannite. Milk remains fluid.

This description corresponds to that of *B. maydis*—an organism

found associated with pellagra by Majocchi,¹ Cuboni,² Paltauf and Heider.³

Macroscopic agglutination tests were made with the serums of seven cases of pellagra, in every case using a strain of bacilli isolated from another patient. This was thought desirable in order to exclude the possibility that the organism was simply accidentally present and caused the formation of agglutinins, although having no connection with the disease. In every case tested, agglutination occurred. The dilutions were as follows: Five cases agglutinated at 1-10, two cases at 1-20. The tubes were allowed to stand for four hours in the incubator and 12-18 hours in the ice box. The organism was not agglutinated except in dilutions of 1-2, by normal serum.

With one exception subcutaneous injections of one half and of one agar slant were harmless to rabbits and guinea-pigs. In the one exception, a rabbit which died in 12 hours, the organs showed only a severe congestion. Although an attempt to isolate a contaminating organism was negative, it is probable that death was caused in this way.

A macacus monkey was fed on bread upon which the bacillus was allowed to grow for 24-48 hours and the feeding continued over a period of six months. The monkey remained healthy and at no time exhibited any gastro-intestinal symptoms.

In three cases of pellagra with dysentery, Shiga bacilli were found. In two cases of pellagra and Shiga bacillus dysentery, the dysentery improved under vaccine treatment, using killed Shiga bacilli, without any effect upon the skin lesions of the patient.

Vaccine treatment with *B. maydis* gave a questionable result in the one case in which it was tried. The patient improved for a time, but later became worse and died. The vaccine was not used in the relapse, owing to the apparent severity of the intoxication.

SUMMARY AND CONCLUSIONS.

The experiments described failed to demonstrate any infectiousness of the blood of pellagrins for the monkey.

¹ Quoted by Sambon, *Brit. Med. Jour.*, 1905, 2, p. 1272.

² *Arch. di Psichiat.*, 1882, 3, p. 353.

³ *Med. Jahrb.*, 1888, 3, p. 383.

In one case the blood of a pellagrin was toxic for the monkey. It is possible, however, that this toxicity was not due to the same toxic agent that was responsible for the symptoms of pellagra.

It is difficult to draw any conclusions from the bacteriological findings. Raubitchek¹ found *B. mesentericus*, an organism closely resembling *B. maydis* culturally, in the stools of pellagrins but found it as well in non-pellagrous control cases. The results of agglutination tests would indicate that the organism was etiologically connected with the disease. Attempts to produce pellagra with it failed in monkeys.

¹ *Wien klin. Wochenschr.*, 1910, 23, p. 903.

THE BACTERICIDAL AND HEMOLYTIC POWERS OF "PARAFFIN" PLASMA AND OF SERUM.*

T. ADDIS.

(From the Laboratory of the Royal College of Physicians of Edinburgh.)

In 1901 Gengou[†] published the details of experiments which showed that plasma had very little or no bacteriolytic action *in vitro* on a variety of organisms which were readily destroyed by serum. This result was advanced as an experimental proof of the correctness of Metchnikoff's theory that complement does not exist in the plasma of the circulating blood but is contained in the white blood corpuscles, and that complement is only found in serum because changes take place in the leukocytes during coagulation which lead to its liberation. Gengou laid stress on the fact that his results were obtained with plasma to which no anticoagulant had been added and which so far at least was in a condition comparable to that in which it exists in the body. This was regarded as a point of importance by some workers, and eight papers have since appeared in which Gengou's experiments were repeated with plasma to which no addition was made. Of these, one, that of Herman, confirms Gengou's results without qualification; the others just as clearly and decisively show that plasma has as much bacteriological and hemolytic power as serum.

On comparing the technic adopted by Gengou with the methods employed by those who have repeated his work, there is found to be one important difference. Gengou prepared mammalian plasma by drawing it directly from a vessel through a paraffined canula into paraffin-lined vessels and centrifuging at 0° C. until it was entirely free from cells. When this plasma was removed from the cooled centrifuge tubes and allowed to attain the ordinary room temperature it quickly clotted. He compared the fluid expressed from this clot with serum derived from the natural coagulation of the whole blood. So in reality he did not directly compare plasma with serum, but the serum from a cell-free plasma with the serum from blood.

Those who deny or confirm the reliability of his results have for the most part adopted another method of procedure. They have used the cell-free plasma itself and not the serum from it. Now this introduces an entirely new factor, the factor

* Received for publication January 17, 1912.

† *Ann. de l'Inst. Pasteur*, 1901, 15, p. 232.

of coagulation. For it may be taken as certain that it is impossible to carry out any bacteriolytic or hemolytic experiment with mammalian paraffin plasma without intercurrent coagulation. This is a point the importance of which appears to a great extent to have escaped the attention it deserves. Thus Dömeny¹ in his paper does not mention coagulation at all. Sweet² states that the plasma coagulated during the course of the experiment. Hewlett,³ who worked with goose plasma, which is much more stable than mammalian plasma, does not say whether it coagulated or not. Löwit and Schwartz,⁴ indeed, recognized the possible influence of this factor and lay no stress on their bacteriolytic work with bird's plasma, since they found that coagulation always occurred. Lambotte,⁵ who also used bird's plasma, says that it coagulated during the bacteriolytic reaction. Herman,⁶ who worked with mammalian plasma, says nothing on this point. Falloise⁷ and Schneider⁸ alone have followed Gengou's method and used the serum expressed from the coagulation of cell-free plasma. Their experiments are the only ones which accurately reproduce Gengou's, and their results are diametrically opposed to his. Nevertheless the faith of the adherents to Metchnikoff's theory is not seriously shaken. They object that Falloise and Schneider during the process of withdrawing the blood and centrifuging it had damaged the leukocytes so that they gave up their content of complement to the plasma. Falloise and Schneider have, indeed, by careful and painstaking work given evidence that the leukocytes were not injured to any appreciable extent, but without avail. Gengou's results are still relied on and it is supposed that by more careful technic he was enabled to obtain a plasma free not only from cells but also from more than traces of leukocytic disintegration. This is a position which has not been made absolutely untenable.

If in a repetition of these experiments the results of Falloise and Schneider were confirmed, the position would have remained for all practical purposes unchanged. The use of plasma itself instead of the serum of plasma is open to the same objections and is further complicated by the possible influence of the process of coagulation on bacteriolysis and hemolysis. Both of these methods were therefore rejected and at Dr. Ritchie's suggestion an attempt, ultimately successful, was made to obtain an unaltered plasma so stable that it would not coagulate during the course of the experiments. Such experiments should, I think, be regarded as conclusive, for the fact that a plasma will remain uncoagulated after being kept for an hour and a half or more at a temperature of 37° C. is in itself conclusive proof that there has been no appreciable amount of damage to the white blood corpuscles. The integrity

¹ *Wien. klin. Wchnschr.*, 1902, 40, p. 105.

² *Centralbl. f. Bakt.*, I, Orig., 1903, 33, p. 208.

³ *Arch. f. exper. Path. u. Pharmac.*, 1903, 49, p. 307.

⁴ *Ztschr. f. Heilkunde*, 1903, 24, pp. 205, 301.

⁵ *Centralbl. f. Bakt.*, I, Orig., 1903, 34, p. 453.

⁷ *Bull. de l'Acad. Roy. de Méd.*, 1905, p. 230.

⁶ *Bull. de l'Acad. Roy. de Méd.*, 1904, p. 157.

⁸ *Arch. f. Hyg.*, 1908, 65, p. 305.

of the leukocytes up to the time when they are removed from the plasma is the essential condition of the continued fluidity of that plasma. When they have previously been injured, even to a very slight extent, the plasma promptly clots. These experiments also make it possible to estimate the effect of coagulation occurring during the bacteriolytic reaction. This is of some importance, for it will allow one to determine the force of the objection to experiments in which this complication occurred.

METHODS.

The preparation of plasma.—The circulating blood remains fluid because of the absence of one of the essential factors in coagulation. This substance, usually termed thrombokinase, is liberated when cells are injured or destroyed. If blood were obtained directly from a vessel without coming in contact with the injured cells of the wound surface and the formed elements in it could be removed without injuring them, a plasma would be obtained which would not spontaneously coagulate. In spite of numerous attempts and of elaborate precautions against damaging the blood cells, no one has as yet succeeded in preparing such a plasma from mammalian blood. On the other hand, with care it is possible with bird's blood. Presumably their blood cells are more resistant to trauma than mammalian blood cells. Such bird's plasma will remain indefinitely fluid if kept at room temperature in a paraffined vessel. In my experience, however, when it is kept in contact with a foreign body at a temperature of 37° C. it will in time coagulate. This indicates that the plasma is not absolutely free from thrombokinase, although the amount must be extremely small, since it is only able to act under the most favorable conditions as to temperature and with the adjuvant action afforded by the presence of a foreign body. Even under these circumstances the period during which the plasma remains uncoagulated is amply sufficient to allow of bacteriolytic or hemolytic experiments being carried out. The real difficulty arises from the fact that this period of fluidity is very much shortened by the addition of bacteria and to a less extent of red blood corpuscles. Their action in this respect is probably a double one. There is the liberation as they become

lysed of any thrombokinase they contain and there is the accelerating action they may exert on the action of the thrombokinase in virtue of their effect as foreign bodies. If they had contained more than traces of active thrombokinase the experiment would have been impossible. But the red blood corpuscles in contradistinction to the leukocytes contain no thrombokinase. If the plasma and leukocytes of the blood from which the corpuscular suspension was prepared were as far as possible removed by washing, it ought to contain only extremely small amount of kinase. A-priori considerations would lead one to expect that kinase would be found in extracts of bacteria, but the existence of a certain degree of specificity in the action of kinase allowed one to hope that it might not induce a rapid coagulation. These considerations made it plain from the commencement that it would be a matter of difficulty to keep the plasma from coagulating during the experiments and as a matter of fact this was found to be the case, and it was only after repeated modifications that a procedure was arrived at by means of which the plasma remained absolutely free from clot for the required time.

The plasma was obtained from cock's blood. The femoral artery was exposed, separated from the tissues of the wound by rubber tissue, and thoroughly washed with warm normal saline. After placing a clip on the proximal end and ligaturing below, the artery was opened and the interior washed. A paraffined glass canula was then introduced and as the clip was removed the canula was pushed into the vessel beyond the part formerly compressed by the clip. After the first few cubic centimeters had run out, the blood was collected in paraffin tubes and as soon as possible centrifuged. This was carried out with a powerful electrical centrifuge. After 10 minutes there was macroscopically a complete separation of the corpuscles from the plasma. The upper part of this plasma was carefully pipetted off, placed in other paraffined tubes and centrifuged again for 20 minutes. Again only the upper part of this plasma was removed and it was this part which was used. Microscopically it was entirely cell free.

In each case part of the blood was collected separately to serve as a source of serum with which to compare the plasma. Some fine

sand was shaken up with it in order to injure the leukocytes and to induce a more rapid coagulation than would otherwise have occurred. There is almost no retraction of the clot in bird's blood and very little serum separates even after centrifuging, but sufficient was obtained by compressing the clot by means of a lead weight.

In the case of plasma or serum used in bacteriolytic experiments all these procedures were carried out under aseptic conditions.

It was found that in the bacteriolytic experiments it was necessary to use tubes lined with sterile block paraffin and closed with paraffined corks, but this precaution could be dispensed with in the hemolytic experiments if care were taken to make the corpuscular emulsion as free as possible from kinase. Rabbit's citrated blood was repeatedly washed by centrifuging in saline solution and at each repetition the upper half of the precipitated corpuscles was removed. In this way an emulsion of red blood corpuscles was obtained which was completely free from white blood corpuscles and platelets. Traces of thrombokinese probably remained adhering to the red cells in spite of washing, but not in sufficient amount to cause coagulation before the hemolytic reaction was ended.

THE COMPARATIVE BACTERIOLYTIC POWER OF PLASMA AND SERUM.

Three series of paraffined tubes were prepared containing respectively plasma, serum, and broth. Progressive dilutions of an emulsion of *B. coli* were added to each and after incubating at 37° C. 0.5 c.c. of each tube was mixed with melted agar and plated. The colonies were counted after 24 hours at 37° C.

Plasma, serum, and broth = 1 c.c.

Emulsion = 2 loops in 10 c.c. broth.

Amount of emulsion or diluted emulsion added = 0.1 c.c.

Time of incubation at 37° C. = 2 hours.

Dilution of Emulsion	No. of Colonies with Plasma	No. of Colonies with Serum	No. of Colonies with Broth
Emulsion	Uncountable	Uncountable	Uncountable
1-10	Uncountable	Uncountable	Uncountable
1-100	1,800	1,740	Uncountable
1-1,000	200	281	2,340
1-10,000	41	55	568
1-100,000	11	8	108

Plasma, serum, and broth = 1 c.c.
 Emulsion = 1 loop in 1 c.c. broth
 Amount of diluted emulsion added = 0.1 c.c.
 Time of incubation at 37° C. = 1½ hours.

Dilution of Emulsion	No. of Colonies with Plasma	No. of Colonies with Serum	No. of Colonies with Broth
1-10	1,680	2,128	Uncountable
1-100	436	264	2,176
1-1,000	105	102	2
1-10,000	13	25	11
1-100,000	0	4	9
1-1,000,000	0	1	7

These results show that bird's plasma has as much bacteriolytic power on *B. coli* as has the serum.

THE COMPARATIVE HEMOLYTIC POWER OF PLASMA AND SERUM.

Advantage was taken of the fact that cock's serum is hemolytic to rabbit's corpuscles.

Time of incubation at 37° C. = 1½ hours.

Corpuscles, 1 per cent suspension	Plasma	Saline	Result	Corpuscles, 1 per cent suspension	Serum	Saline	Result
1 c.c.	0.07 c.c.	0.93 c.c.	C	1 c.c.	0.07 c.c.	0.93 c.c.	C
1 c.c.	0.06 c.c.	0.94 c.c.	C	1 c.c.	0.06 c.c.	0.96 c.c.	+++
1 c.c.	0.05 c.c.	0.95 c.c.	++	1 c.c.	0.05 c.c.	0.95 c.c.	++
1 c.c.	0.04 c.c.	0.96 c.c.	+	1 c.c.	0.04 c.c.	0.96 c.c.	+
1 c.c.	0.03 c.c.	0.97 c.c.	0	1 c.c.	0.03 c.c.	0.97 c.c.	0

Time of incubation at 37° C. = 1 hour.

Corpuscles, 1 per cent suspension	Plasma	Saline	Result	Corpuscles, 1 per cent suspension	Serum	Saline	Result
1 c.c.	0.15 c.c.	0.85 c.c.	C	1 c.c.	0.15 c.c.	0.85 c.c.	C
1 c.c.	0.1 c.c.	0.9 c.c.	++++	1 c.c.	0.1 c.c.	0.9 c.c.	C
1 c.c.	0.09 c.c.	0.91 c.c.	++++	1 c.c.	0.09 c.c.	0.91 c.c.	C
1 c.c.	0.08 c.c.	0.92 c.c.	++++	1 c.c.	0.08 c.c.	0.92 c.c.	++++
1 c.c.	0.07 c.c.	0.93 c.c.	++	1 c.c.	0.07 c.c.	0.93 c.c.	+++
1 c.c.	0.06 c.c.	0.94 c.c.	+	1 c.c.	0.06 c.c.	0.94 c.c.	—
1 c.c.	0.05 c.c.	0.95 c.c.	+	1 c.c.	0.05 c.c.	0.95 c.c.	—
1 c.c.	0.04 c.c.	0.96 c.c.	0	1 c.c.	0.04 c.c.	0.96 c.c.	+
1 c.c.	0.025 c.c.	0.975 c.c.	0	1 c.c.	0.025 c.c.	0.975 c.c.	0

As regards hemolytic power, therefore, no noticeable distinction was apparent between plasma and serum. The slight differences which were found lie well within the limits of error of the method.

THE EFFECT OF INTERCURRENT COAGULATION ON BACTERIOLYSIS AND HEMOLYSIS WITH PLASMA.

In the above experiments there was no trace of coagulation in the plasma at the end of the time of incubation. In some experiments, however, coagulation did occur and the question arose as

to whether this process had in any way modified the bacteriolysis or hemolysis. When the plasma had coagulated it was of course impossible to plate it, and there was therefore no means of measuring accurately the degree of bacteriolysis which had occurred. But a rough idea was obtained by making cultures on agar from the clotted plasma, serum, and broth, in order to see with what dilution of emulsion the plasma or serum had become sterile. This method did not give constant results, but no indication was obtained that the clotting had either helped or hindered the bacteriolysis.

It was however easy to determine the influence of coagulation on hemolysis. Three series of tubes containing plasma and a suspension of corpuscles were prepared. In the first, coagulation was induced in the plasma by the addition of thrombokinase (a dilute watery testicular extract) before the corpuscles were added. They were of course unable to mix with the clot and formed a separate layer above it. In the second series coagulation was induced during the reaction by the addition of thrombokinase. In the third series instead of thrombokinase the same amount of water was added and the plasma remained throughout uncoagulated. Controls showed that the thrombokinase had no hemolytic action. It was necessary to use as a diluting fluid for the corpuscles and for the plasma a saline solution containing 0.06 per cent of calcium chloride, as otherwise the tubes containing the smaller amounts of plasma did not coagulate with kinase, because the calcium concentration had in them been reduced below the minimum amount which is necessary.

Rabbit corpuscles = 1 per cent suspension in a solution containing 0.9 per cent NaCl and 0.06 per cent CaCl.

Saline solution = 0.9 per cent NaCl and 0.06 per cent CaCl.

Thrombokinase = 0.1 c.c.

Time of incubation = 1½ hours.

PLASMA COAGULATED BEFORE ADDITION OF CORPUSCLES				PLASMA COAGULATED DURING HEMOLYSIS				PLASMA UNCOAGULATED			
Corp.	Plasma	Sodium	Result	Corp.	Plasma	Saline	Result	Corp.	Plasma	Saline	Result
1 c.c.	0.1 c.c.	0.9 c.c.	Hemolysis	1 c.c.	0.1 c.c.	0.9 c.c.	C	1 c.c.	0.1 c.c.	0.9 c.c.	C
1 c.c.	0.09 c.c.	0.91 c.c.	"	1 c.c.	0.09 c.c.	0.91 c.c.	++++	1 c.c.	0.09 c.c.	0.91 c.c.	++++
1 c.c.	0.08 c.c.	0.92 c.c.	"	1 c.c.	0.08 c.c.	0.92 c.c.	++	1 c.c.	0.08 c.c.	0.92 c.c.	++
1 c.c.	0.07 c.c.	0.93 c.c.	"	1 c.c.	0.07 c.c.	0.93 c.c.	+	1 c.c.	0.07 c.c.	0.93 c.c.	+
1 c.c.	0.06 c.c.	0.94 c.c.	o	1 c.c.	0.06 c.c.	0.94 c.c.	o	1 c.c.	0.06 c.c.	0.94 c.c.	o

The result was thus the same in each case. Whether the plasma was coagulated before or during the hemolysis or remained uncoagulated, 0.07 c.c. produced some hemolysis and 0.06 c.c. caused none. In the first case, where the clot and corpuscles remained separate, the degree of hemolysis was difficult to estimate, but there was no doubt about the last tube showing lysis, for the least trace of hemoglobin could be seen diffusing into the clear saline solution left by the settling of the corpuscles onto the clot.

Coagulation has therefore no effect on hemolysis.

THE EFFECT OF THE CLOT ON THE COMPLEMENT-CONTENT OF THE SERUM.

A possible objection to the above comparative experiments with plasma and serum is the fact that before testing they were not subjected to exactly the same conditions. The serum was in contact with the clot, which might conceivably increase or diminish the amount of complement. Indeed, Ainly Walker¹ and Henderson Smith² have described slight changes in the amount of bacteriolytic complement of serum separated at different intervals of time from the clot. In general they maintain that there is a gradual increase in complement for the first five or six hours, which they ascribe to a slow liberation of complement from leukocytes entangled in the fibrin. The later diminution which occurred is referred to absorption of complement by the clot. The serum in the above experiments was removed from contact with the clot after an interval varying from three to six hours, so that according to Ainly Walker and Henderson Smith it should have contained about its maximum amount of complement. But bird's blood has not been investigated as regards this point, and it seemed advisable to do so, since it was possible that a rapid absorption of complement by the clot might be the cause of the serum containing no more complement than plasma.

¹ *Jour. Hyg.*, 1903, 3, p. 52.

² *Proc. of the Roy. Soc.*, 1906, Series B, 79, p. 378.

RABBIT'S CORP. 1 PER CENT	BIRD'S SERUM	TIME DURING WHICH THE SERUM HAD BEEN IN CONTACT WITH THE CLOT					
		$\frac{1}{2}$ Hour	1 $\frac{1}{2}$ Hours	2 Hours	3 Hours	4 Hours	6 Hours
1 C.C.	0.00 C.C.	C	C	C	C	C	C
1 C.C.	0.05 C.C.	++++	++++	++++	++++	++++	++++
1 C.C.	0.07 C.C.	++++	++++	++++	++++	++++	++++
1 C.C.	0.06 C.C.	++++	++++	++++	++++	++++	++++
1 C.C.	0.05 C.C.	++++	++++	++++	++++	++++	++++
1 C.C.	0.04 C.C.	++	++	++	++	++	++
1 C.C.	0.03 C.C.	+	+	+	+	+	+
1 C.C.	0.02 C.C.	o	o	o	o	o	o

There was thus no noteworthy difference in the hemolytic power of serum removed at these various intervals of time from the clot, and one may conclude that in bird's blood the clot has no effect on the amount of complement, within the first six hours at least. In view of the apparent difference between bird's blood and mammalian blood the same experiment was repeated with rabbit's serum.

A rabbit was bled, and after mixing the blood was poured into eight centrifuge tubes and allowed to clot. At various intervals thereafter each tube was centrifuged to separate the serum, and the amount of complement in it was determined.

Corpuscles = 1 per cent suspension of ox-blood corpuscles.

I.B. = serum of rabbit immunized against ox-blood corpuscles.

CORP.	I.B.	COMPLEMENT	TIME DURING WHICH THE COMPLEMENT HAD BEEN IN CONTACT WITH THE CLOT						
			$\frac{1}{2}$ Hour	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	8 Hours
I C.C.	0.005 C.C.	0.2 C.C.	C	C	C	C	C	C	C
I C.C.	0.005 C.C.	0.1 C.C.	+++++	+++++	+++++	+++++	+++++	+++++	+++++
I C.C.	0.005 C.C.	0.05 C.C.	+++++	+++++	+++++	+++++	+++++	+++++	+++++
I C.C.	0.005 C.C.	0.04 C.C.	+++++	+++++	++	++	++	++	+++++
I C.C.	0.005 C.C.	0.03 C.C.	++	++	+	+	+	++	++
I C.C.	0.005 C.C.	0.02 C.C.	+	+	o	+	+	+	+
I C.C.	0.005 C.C.	0.01 C.C.	o	o	o	o	+	+	+
I C.C.	0.005 C.C.	0.005 C.C.	o	o	o	□	o	o	o

Here also there was no appreciable variation, and I have thus been unable to confirm the statements as to variations in the amount of complement in serum, either with bird's or with rabbit's serum.

CONCLUSIONS.

Unaltered bird's plasma has as much bacteriolytic and hemolytic power as serum. This equality cannot be accounted for by supposing that the clot has absorbed a large part of the complement in the serum, since it has been shown that no such absorption occurred.

Nor, on the other hand, can these results be put aside as inconclusive because of the possibly complicating effect of coagulation, since no coagulation took place. They show that the hypothesis that complement is derived from the leukocytes when they are injured is not correct. For if the white blood corpuscles had been damaged even slightly, thrombokinase would have been liberated and the plasma would have coagulated. No more delicate test of the degree of leukocytic injury than the length of time during which a plasma will remain fluid can be devised. Gengou maintains that the amount of complement in plasma is proportional to the degree of leukocytic disintegration. Yet in bird's plasma, in which, as has been shown, there was less cell injury than in his plasma, there was nevertheless as much complement as in serum, whereas on his hypothesis there should have been scarcely a trace.

Once the alleged greater accuracy of Gengou's technic is disproved, the matter becomes one to be decided from the evidence of results. This evidence is very much in favor of the view that complement exists in the circulating plasma and that it is not derived from injured leukocytes. Gengou's results have indeed been confirmed by one observer, but as he worked with very small amounts of plasma and serum, with hanging drop preparations of plasma and serum for example, there are possible sources of error which might explain his findings. The seven others who have investigated this point are unanimous in coming to the conclusion that plasma contains as much complement as serum. Their results are none the less to be accepted as valid evidence canceling that brought forward by Gengou, although coagulation may have taken place in the plasma during the experiments, since I have shown that the process of coagulation has no influence on the course of bacteriolytic or hemolytic reactions. And when, as in the experiments described in this paper, it is shown that plasma derived from blood in which the amount of cell injury has been minimal contains as much complement as serum from blood in which every influence favored the destruction of the leukocytes, I think it is no longer possible to hold that complement is derived from injured leukocytes. This, then, is another point to be added to all the other evidence which goes to show that complement is present in the circulating plasma.

OBSERVATIONS ON BACILLUS MESENTERICUS AND ALLIED ORGANISMS.*

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THE MAIN TYPES OF THE SUBTILIS GROUP.

The frequency of finding distinct variations in the morphology and cultural characters of the organisms commonly classed as *B. subtilis* led to the study of as many of these forms as could be obtained, with the object of determining if there were any particular relations between them, or any constancy in their characters.

The number of spore-bearing organisms which possess feeble or no pathogenic properties is considerable. In the literature there occur at least 43 distinct species. Of these, however, a considerable proportion must be regarded as mere variants of one or other well-marked types, even though they were originally found in habitats quite disconnected with a bacteriological laboratory.

It is convenient to divide the organisms of this class into four or perhaps five standard types. These types are respectively the bacillus subtilis, the bacillus megatherium (De Bary), the bacillus vulgatus (Flügge), the bacillus mesentericus (Flügge), and possibly the bacillus ellenbachensis (Stutzer).

Morphology of these types.—*B. subtilis*, as is well known, varies considerably in its appearance, though the typical forms are square-ended rods. It is usually smaller than *B. anthracis*, but otherwise very similar. The spores, however, though central, are either of the same diameter or a little larger than the bacillus. Other forms occur in which the bacillus is shorter and thicker, and either square-ended or rounded at the ends. In such cases the spore occupies nearly two-thirds of the length of the organism, and is conspicuously broader. Other forms again occur, apparently identical in cultural reactions, which are still shorter, and usually show a very

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characteristic form owing to the large size of the spore, which then appears to have a cap upon each pole corresponding to the remains of the original bacillus.

B. megatherium differs from the preceding in its slender form, invariably with square ends, and in frequently having a narrow elongated spore toward one pole. The spore is not central, but its center is at some point between the center of the bacillus and its pole. In a few instances the spore is quite terminal, and when its diameter is larger than that of the bacillus, the whole structure acquires a form not unlike that of the bacillus of tetanus, and very like that of the bacillus of malignant edema. As a rule the spore is of exactly the same diameter as the bacillus which bears it. This form may show a granular structure—a false beading—when stained by Gram's method.

B. vulgatus parades under several other names. *B. mesentericus vulgatus* is agreed to be the same organism, and it has also been called the potato bacillus. This organism is long and slender, and either square-ended or rounded at the ends. Except that long thrixlike forms may occur, and that it is more slender than the preceding, the characters of the two are practically identical.

B. mesentericus presents variations of morphology which on the one hand may be subtiliform, and on the other, megatheriform. As a rule it occurs as a short bacillus with rounded ends and a central spore which is broader than the organism and oval in shape. A form is quite common in which the spore is so large that the bacillus itself is reduced to a cap at each end.

The Ellenbach bacillus goes by a number of other names, having been described variously by different authors (Burchard, Frankland, Flügge, Kern, Russell). The ends are rounded, and there are numerous clear spots in the length of the bacillus. It is as large as or larger than the anthrax bacillus, and the oval spores are larger compared with it. It may be arbitrary to describe it thus as a separate main type, but the beaded appearance is so characteristic a feature that it may be emphasized in this way. In the course of our work we have come across gram-positive organisms which were regarded by others as diphtheroid, until we were able to demonstrate the spore formation.

Attached to each of these types there are numerous species described in the literature. They may be arranged as follows:

Under *B. subtilis*: *B. bernesis*, *B. carotarum*, *B. simplex*, *B. sessilis*, *B. leptosporus*, *B. peptonificans*, *B. tenuis*, *B. aterrimus*, *B. implexus*, *B. loxosporus*, *B. natans*, *B. vacuolus*.

Under *B. ellenbachensis*: *B. petroselini*, *B. cereus*, *B. limosus*, *B. lutulentus*, *B. cursor*, *B. loxosus*, *B. turgescens*, *B. stoloniferus*, (*B. robur*).

Under *B. megatherium*: *B. petasites*, *B. silvaticus*, *B. hessii*, *B. quercifolius*, *B. lactis*, *B. pumilus*, *B. leptodermis*.

Under *B. vulgatus*: *B. graveolens*, *B. butyricus*, *B. tumescens*, *B. gummosus*, *B. levaniformis*, *B. gelatinosus betae*, *B. spongiosus*, *B. geniculatus*, *B. granulatus*, *B. lactis*, *B. lacticola*, *B. goniosporus*, *B. lacteus*, *B. aureus*, *B. cylindrosporus*, *B. amarificans*, *B. agglomeratus*, *B. lutulentus*, *B. sphericus*, *B. gracilis*, *B. pseudo-tetanicus*, *B. albuminis*.

Under *B. mesentericus*: *B. mesentericus ruber*, *B. teres*, *B. filiformis*, *B. liodermos*, *B. pumilus*, *B. ruminatus*, *B. albolactis*, *B. tomentosum*, *B. pansini*, *B. mucosus* (Zimmermann).

THE VARIOUS FORMS OF GROWTH UPON AGAR AND POTATO.

For the purpose of introducing clearness into a difficult group we may arrange the above organisms according as they fall into the types named, but in doing so it must be conceded that the morphology becomes the sole guide, and that this, along with the cultural characters, may be rendered unreliable owing to the variability which is so frequent in individual species. Different critics could easily arrange the various species differently. It is more simple to divide the organisms into *three* groups according to the mode of action upon *sugar media* (see below), but in view of the characteristic appearances presented by the slope cultures (potato or agar) a critical study of such points may be dealt with first, considering that Lehmann and Neumann regarded it best to classify these *subtilis* forms according to the growth on potato.

The *growths upon agar*, as studied in this laboratory, may be divided into these well-marked forms:

a) A white growth which spreads more or less rapidly over the surface of the agar, so that within 48 hours the whole is covered. In this form the growing margin is leaflike and acanthiform. The surface is dry, and frequently has a powdery appearance, as if dusted over with flour. The growth is opaque. In one or two

instances the growth was scanty, dead chalk-white in color, and slow of development (cretaceous form).

Examples—Powdery growth: *B. subtilis*, *B. megatherium*, *B. butyricus*, *B. oxalaticus*, *B. parvus*.

b) A moist creamlike growth which is rapid and very abundant. This form tends to undergo pigmentation, developing a creamy-brown color in some instances, a fawn color in others, and a brilliant ocher-yellow color in others.

c) A gummy transparent growth, which is very easily identified. This form has exactly the appearance of a thin film of ice lying over an opaque milky deposit. This icelike or glassy surface is striking.

Examples of b and c—Smooth growth: *B. mesentericus*, *B. bernensis*, *B. tumescens*, *B. granulatus*, *B. petasites*, *B. asterosporus*, *B. pumilus*, *B. simplex*.

d) A fawn-colored, opaque, dry, rather scanty growth. This form may show fine polygonal depressions or "reticulations" of its surface, the general growth being flat. The older portions of this growth are powdery in appearance.

Examples Reticulate growth: *B. mesentericus ruber*, some forms of *B. mesentericus*, some forms of *B. megatherium*.

e) A wrinkled growth. These wrinkles occur in various forms, passing in different examples from a fine polygonal, fawn-colored, slightly raised deposit (see *d*), through a more abundant and more raised fawn-colored growth (see *b*), which covers a large portion of the culture tube (though most marked at the lower end), to an excessively abundant wrinkling, such as occurs in some smegma bacillus cultures, and occasional tubercle bacillus cultures. Strikingly picturesque growths may be produced in such cases. The wrinkling, however, may not be dry, but it may possess a luscious moist appearance, especially when arising in the moist forms of creamy or milky growth. Here the wrinkles are usually much larger, and more widely separated.

Examples—Wrinkled growth: *B. vulgatus*, *B. graveolens*, *B. fusiformis*, *B. teres*, *B. liodermos*, *B. mesentericus*, *B. tenuis*, *B. ellenbachensis*, *B. ruminatus*.

These different variations of aspect are obviously not hard and fast, but there are gradual transitions met with between one and

another. Not only are such transitions found in studying series of specimens from different sources, but the same organism may be seen to undergo changes in the courses of successive planting upon the same medium. Thus, in a form which at the outset possessed a growth like the fourth variant, we were able to demonstrate a change through the second to the third by planting at monthly, or two-monthly, intervals—the organisms having been kept in the incubator for three or four weeks at a time. In four months the organism had developed the third form. In another case, the central parts became like type *b*, while the periphery was still like type *c*. Such changes were noticed in several cases, and in numerous examples we noticed that the particular culture would alter in aspect as the medium became more and more dry, though in such examples the change was evidently due to the increased spore production, which invariably predisposes to a dry, powdery, or dusty appearance.

These remarks will indicate what we wish to emphasize, namely, that cultural characters of the “*subtilis* group” are not fixed in any of its members, so that it is not necessarily incorrect to call a *B. mesentericus*, *B. subtilis*, or vice versa. There is little real criterion to go upon. This statement, however, requires qualifying by indicating that *sugar reactions* remain fairly constant.

The *growths upon potato* may be classified thus:

a) Pigmented growth. These growths are usually moist and vary from a fawn or yellowish brown through ocher to rose or pink. Such growths are elevated and have a granular surface. Sometimes they are wrinkled. The pigment may require several days for its development.

1. Fawn color: *B. pisiformis* (coliform), *B. asterosporus* (no folds), *B. teres* (dry surface).

2. Pale yellow: *B. luteus*, *B. petasites*.

3. Ocher yellow: *B. ochraceus* (proposed name).

4. Yellowish brown: *B. parvus*.

5. Reddish brown: *B. silvaticus*.

6. Pink or rose: *B. tenuis* (no wrinkling), *B. mesentericus ruber* (wrinkles late).

b) Non-pigmented dry growth. The surface in such cases is

dry and dusty (*B. subtilis*, *B. tenuis*). Other varieties have been found which we propose to name according to their main character: *B. iridescens*, *B. cretaceus communior* (abundant growth), *B. cretaceus infrequens* (scanty growth).

c) Non-pigmented moist growth. The surface may present a gummy appearance, with folds arising (*B. simplex*, *B. ruminatus*) or without folds (*B. liodermos*). The surface may be creamy; in such cases the folds may be much raised (*B. vulgatus*, *B. graveolens*, *B. aterrimus*); in other cases the growth has a reticulated surface (*B. mesentericus*); in others the creamy deposit is quite smooth (*B. pumilus*). The surface may be coliform (*B. oxalaticus*, *B. tumescens*, *B. megatherium*, *B. butyricus*) or dull gray (*B. ellenbachensis*, *B. subtilis*, *B. mesentericus*, *B. ruminatus*).

This method of grouping affords a convenient guide to the identification of some of these organisms, but, as already indicated, is not rigidly adhered to by the respective species.

SUBDIVISION OF THE MEMBERS OF THIS GROUP ACCORDING TO MORPHOLOGY.

The variants in cultural forms would naturally be assumed to go hand in hand with morphological variations, but this is not strictly speaking true. The powdery forms are usually long slender or long thick bacilli, while the gummy forms are usually shorter and rounded. In other words, the former are subtiliform, and the latter mesentericiform. But many of the gummy or glassy forms do show delicate slender rods with terminal or nearly terminal spores, such as occur in *megatherium* and *vulgatus*.

The moist creamy forms (*b*) are usually long rods of variable thickness and conform to the *megatherium* type, though here again we meet with dry wrinkled forms of type *c* conforming to the same morphological type.

Among the chalky forms we have met with two examples which were extremely striking. In one the growth on the agar plate was very beautiful, having a mathematically regular feathery star form, the limbs of the star being gently curved. In another form the growth was very scanty, and possessed an extremely white, opaque,

powdery aspect. Both forms were isolated from the intestinal contents of caterpillars.

Classifying the brown species according to the differences in morphology, we have:

Rods, decidedly longer than broad—square ends: *B. robur* (Meyer and Neide), *B. megatherium*, *B. vulgatus*, *B. carotarum* (Koch), *B. mycoides*, *B. mesentericus ruber*, *B. sphericus*, *B. silvaticus*, *B. parvus*—in order of size.

Rods, decidedly longer than broad—rounded ends: *B. subtilis*, *B. ellenbachensis*, *B. simplex*, *B. petasites* (Meyer and Gottheil), *B. pumilus* (Meyer and Gottheil), *B. bolear* (Schiff-Georgini), *B. ruminatus* (Meyer and Gottheil), *B. teres* (Meyer and Neide), *B. graveolens*, *B. mesentericus*, *B. asterosporus*—in order of size.

Rods, very little longer than broad: cocci-bacillary forms; some *B. teres*, and *B. fusiformis*.

Motility. Gram-staining.—All the forms are gram positive and sluggishly motile by the aid of peritrichous flagella. There are a few exceptions to the latter rule, in that *B. sphericus*, *B. fusiformis*, and *B. asterosporus* are very actively motile, while young cultures of *B. subtilis*, *B. parvus*, *B. silvaticus* are fairly motile, and *B. ellenbachensis*, *B. mesentericus ruber*, *B. tenuis*, *B. implexus*, *B. oxalaticus*, *B. simplex*, *B. carotarum* and *B. ruminatus* are non-motile.

ACTION ON GELATIN AND MILK.

Action on gelatin.—The group reaction is liquefaction after varying periods of time. Some specimens liquefy in three or four days. In our series we have found that some forms do not liquefy within three weeks.

Action on milk.—This is quite typical. It consists in the conversion, within a few hours, of the opaque fluid into a watery translucent or even transparent liquid, with a flocculent yellowish or pale brown precipitate. The fluid turns an ocher or orange or lemon yellow, or, occasionally, a violet, or even blue-black color, which is very striking. Some forms produce acid (*B. gastrophilus*) and some produce a preliminary coagulation—*B. anthracis*, *B. vulgatus* (occasionally), *B. ellenbachensis*, *B. ruminatus*, *B. burnensis*,

B. butyricus, *B. asterosporus*, *B. oleac*. In the case of *B. pumilus* and *B. petasites*, coagulation occurs quite late.

VARIATIONS OF ACTIONS ON SUGARS.

Action on sugar media.—Most of the published observations are very incomplete. Dextrose may be fermented with acid production in some instances (*B. tenuis*, *B. bernensis*, *B. teres*, and *B. fusiformis* have no action on dextrose) while lactose is rarely attacked (only by *B. butyricus*). The other sugars have been systematically tested in our series, which includes examples obtained from the intestinal contents of both vertebrates and invertebrates—the snail, various caterpillars, frogs, snakes, perch, bass, rats, and some poultry. In addition, many forms were found in the material spoken of at the time as “Chinese eggs,” besides examples from various samples of well-water and various forms found in the hospital patients.

DEXTROSE: This sugar was invariably fermented, though to varying degrees. Using the Andrade indicator and using an arbitrary color scale in which 100 represents the maximum coloration produced by any organism, the acid formation was represented by figures varying from one to 40. In seven cases an appreciable amount of gas was formed (Nos. 4, 17, 23, 25, 27, 33, 35).

Time relations: As a rule the acidity did not alter after the second day, but in some instances there was a marked increase of acidity on the fourth and fifth days of incubation to be followed by a fall (*B. petasites*, *B. megatherium*, *B. tumescens* [No. 11]). In one case the maximum acidity was on the third day (*B. tumescens* [No. 11]).

LACTOSE: This was invariably unaffected in the organisms classed into groups 1 and 2, but in one or two forms corresponding to the *B. megatherium* type, both acid and gas were produced to a moderate extent, but only after six or seven days' incubation (*B. teres*).

SACCHAROSE: This sugar was unaffected by organisms of the first group. They may be termed *non-saccharose fermenters*. The second group comprises those which invariably showed a fermentation with acid production alone, a change presumably due to inversion

of the saccharose. Gas was not formed. Some of the specimens gave a reaction within a day or two, while others failed to give a reaction within two, or sometimes three, weeks. In the case of a third group the saccharose was fermented with both acid and gas production, though in varying and small amounts.

TABLE 1.
NON-SACCHAROSE FERMENTERS.

Consec. No.	Name of Organism	Source	Dextrose	Lactose	Saccharose	Maltose	Mannite	Levulose	Dextrin
				A G			A G		A G
1	<i>B. subtilis</i>	Canned eggs	3-0	0	0	0	0	0	—
2	<i>B. subtilis</i>	Human jejunum	3-bubble	0	0	0	0	0	—
3	<i>B. mesentericus ruber</i>	Caterpillar	25-0	0	0	0	0	0	5-
4	<i>B. ochraceus</i>	"	40-25	0	0	0	10-0	0	5-15
5	<i>B. megatherium</i>	Bass	5-0	0	0	0	0	0	—
6	<i>B. gummosus</i>	Snail	35-0	0	0	0	0	0	—
7	<i>B. gummosus</i>	"	15-0	0	0	0	0	Trace 0	—
8	<i>B. ruminatus</i>	Caterpillar	30-0	0	0	0	0	0	2-0
9	<i>B. ruminatus</i>	"	30-0	0	0	0	0	10-15	—
10	<i>B. petasites</i>	Canned eggs	10-0	0	0	0	0	0	—
11	<i>B. tumescens</i>	Snail	25-0	0	0	0	0	0	—
12	<i>B. liodermos</i>	Canned eggs	20-0	0	0	0	0	0	—
13	<i>B. gastrophilus</i>	Hen's egg	10-5	0	0	0	0	—	0-1

TABLE 2.
SACCHAROSE FERMENTERS.

Consec. No.	Name of Organism	Source	Dextrose		Lactose		Saccharose		Maltose		Mannite		Levulose		Dulcite	Dextrin
			A G	A G	A G	A G	A G	A G	A G	A G	A G	A G	A G	A G		
14	<i>B. mesentericus</i>	Bass intestine	15-0	0	5-0	0	5-0	0	5-0	0	5-0	0	5-0	0	0	0
15	"	Canned egg	15-0	0	10-0	0	5-0	0	5-0	0	5-0	0	5-0	0	0	0
16	"	"	5-0	0	5-0	0	5-0	0	5-0	0	5-0	0	5-0	0	0	0
17	"	Perch intestine	10-0	0	30-15	0	20-0	0	20-0	0	20-10	0	20-10	0	0	0
18	<i>B. cretaceus</i>	Raw sugar	3-0	0	5-0	0	Trace 0	0	Trace 0	0	Trace 0	0	Trace 0	0	0	0
19	<i>B. megatherium</i>	Rat intestine	15-0	0	15-0	0	0	0	0	0	0	0	0	0	0	0
20	"	Bass intestine	5-0	0	5-0	0	0	0	0	0	0	0	0	0	0	0
21	<i>B. gummosus</i>	Snail intestine	35-0	0	35-0	0	0	0	0	0	0	0	0	0	0	0
22	<i>B. teres</i>	Perch intestine	10-10	0	Late	0	0	0	0	0	0	0	0	0	0	0
23	<i>B. petasites</i>	Caterpillar	45-25	3-0	3-0	0	10-0	0	10-0	0	10-0	0	10-0	0	—	5-15

Time relations: Acid production became marked on the fourth day, and reached its maximum during the fifth and sixth days.

(Examples: *B. megatherium*, *B. mesentericus*, *B. teres*, *B. cretaceus*.)

MALTOSE: This was rarely attacked, but a few variants were found (Nos. 24-31, 33-35) in which a slight degree of acid formation

was noticed. These forms occurred indiscriminately in the three groups referred to. Some megatherium forms produced gas as well as acid.

Time relations: Gas was produced by some in considerable quantity, the maximum formation occurring on the fourth day, though acid was formed in greater and greater quantities from this date onward till the fourteenth day, when it diminished again (*B. megatherium*). As a rule the reactions remained constant from the time at which they first appeared (first day).

MANNITE: The mannite fermenters were very few. Some produced only acid, and some both acid and gas.

Time relations: Acid formation steadily progressed till the 12th day, after which diminution occurred (*B. megatherium* No. 32). As a rule the reactions remained the same throughout as they were on the first day.

Summarizing these sugar reactions for diagnostic purposes, we notice that the first broad subdivision of the members of the group is into (a) fermenters of dextrose; (b) non-fermenters of dextrose.

The second group is the less numerous, including *B. subtilis*, *B. tenuis*, *B. fusiformis*, *B. atterimus*; *B. gummosus*, *B. gastrophilus* may also reasonably fall into this group. It is important to note that *B. subtilis* is much more frequently a non-fermenter than a fermenter and that its action is always minimal, even if present.

The first group may be divided up into single-sugar fermenters or multiple-sugar fermenters, and the latter into saccharose fermenters, mannite fermenters, maltose fermenters, and levulose fermenters.

Multiple fermenters:

a) Saccharose fermenters: *B. megatherium*, *B. mesentericus*, *B. gummosus* (marked), *B. teres* (late), *B. cretaceus* (variable).

b) Mannite fermenters: *B. mesentericus* (some forms), *B. petasites*, *B. ochraceus*.

c) Maltose fermenters: *B. megatherium*, *B. pumilus*, some forms of *B. mesentericus*, *B. gastrophilus*.

d) Levulose fermenters: *B. ruminatus*, some variants of *B. mesentericus*.

The other organisms are all "single-sugar fermenters," and only produce a minimal reaction. *B. mesentericus ruber*, however, acts vigorously.

TABLE 3.
MULTIPLE FERMENTERS.

Consec. No.	Name of Organism	Source	Dextrose		Lactose		Succharose		Maltose		Mannite		Levulose		Dextrin		Dulcite
			A	G	A	G	A	G	A	G	A	G	A	G	A	G	
24.....	<i>B. megatherium</i>	Bass intestine	5-	0	0	0	10-15	25-10	0	0	0	0	—	—	—	—	0
25.....	"	"	15-15	5-	0	0	20-10	10-0	0	0	0	0	—	—	—	—	0
26.....	"	Snail	20-0	0	0	0	70-0	0	0	0	0	0	15-0	—	—	—	0
27.....	"	"	10-10	10-10	5-	0	15-15	0	0	0	0	0	—	—	—	—	0
28.....	"	Child, feces	5-	0	0	0	15-0	15-0	0	0	0	0	—	—	—	—	0
29.....	"	"	10-2	15-	0	0	5-6	3-2	0	0	0	0	—	—	—	—	0
30.....	"	Caterpillar	25-3	0	0	0	2-0	0	0	0	0	0	—	—	10-0	—	0
31.....	"	Bass intestine	3-	0	0	0	3-0	5-0	0	0	0	0	—	—	—	—	0
32.....	"	Snail	10-0	0	0	0	0	0	0	0	20-0	15-0	—	—	—	—	0
33.....	<i>B. mesentericus</i>	Bass intestine	0	0	0	0	10-10	5-10	—	—	—	—	—	—	—	—	0
34.....	<i>B. gastrophilus</i>	Canned egg	5-	0	10-	0	0	5-0	0	0	0	0	—	—	—	—	0
35.....	<i>B. pumilus</i>	"	0	0	0	0	75-10	5-0	—	—	—	—	—	—	5-0	—	0

TABLE 4.
NON-FERMENTERS OF DEXTROSE.

- 36.....*B. subtilis*, in six examples
 37.....*B. tenuis*, in three examples
 38.....*B. cretaceus*
 39.....*B. gummosus*
 40.....*B. fusiformis*
 41.....*B. gastrophilus*
 42.....*B. aterrimus*
 43.....*B. bernensis*
 44.....*B. teres*

The occurrence of motile and non-motile forms, the occurrence of milk-coagulators and milk non-coagulators, as well as the presence of morphological variations, prevent the use of the above classification as the main measure of identification of these organisms. It is for this reason that a diagnostic table which combines all such considerations has been devised (see p. 223).

LEVULOSE: Very strong action was noticed with levulose in a number of instances. The medium became strongly acid, and in a few instances gas was also produced. Examples: Nos. 7, 9, 17, 26, 32.

Time relations: This was often late, the change commencing on the fourth day and reaching a maximum on the seventh day (*B. ruminatus* No. 9).

DULCITE: In no instance was there any action on dulcite.

Time relations: If any change occurred at all it was in the form of gas production, appearing after seven days' incubation, but without the formation of acid (*B. ruminatus* No. 9).

DEXTRIN: Five specimens showed acid formation.

Time relations: Occasionally gas was formed after seven days' incubation (*B. petasites* No. 23).

OTHER BIOLOGICAL PROPERTIES.

Indol formation.—The formation of indol in peptone water was noticed in a few instances, and when present was very decided. There was no regularity of action in relation to the grouping according to sugar fermentation, typically in *B. mesentericus*, *B. ochraceus* (in one example), *B. mesentericus* (in three examples), *B. megatherium* (in one), *B. simplex* (in one), *B. subtilis* (in three).

Nitrites.—The formation of nitrites was equally irregular, and was not always met with in the same examples as was the production of indol. *B. tenuis* (two examples), *B. cretaceus* (two), *B. mesentericus* (two), *B. fusiformis* (one), *B. gastrophilus* (one), *B. vulgatus* (three).

Resistance to ordinary agents.—It was found that the resistance of the spores of these bacilli was remarkably high. This was proved by difficulties in getting rid of the organisms by the ordinary methods of sterilization. The only method of destroying the spores with certainty was by dry heat to 120° C. for a quarter to half an hour. Autoclaving was not successful unless repeated five or six times. As regards the sterilizing of exploratory syringes which had become contaminated with such organisms, it was found that prolonged boiling in carbolic lotion was necessary.

Optimum temperature.—As a rule the organisms flourish best at 30° C., but they will grow rapidly at room temperature. They grow very rapidly in the incubator, the whole surface of the agar being covered within 12 hours in many instances.

DISTRIBUTION IN NATURE.

It is hardly necessary to state that the members of this group are very widespread, but the wide distribution of the *varieties*

named is not so generally understood. Thus we have found them in throat cultures as well as in throat swabs, and we have also obtained variants of *B. mesentericus* in the lymph glands of the groin both in children and adults in the general hospital wards. Excluding technical errors we have come to consider that such organisms may occur in individuals suffering from no disease with which such an organism could have a causal relation.

That false interpretation could be placed on such findings is shown by the report of a "cancer bacillus" by Sheurlen which belonged to this group. There seems little doubt that the greatest precautions will not prevent the discovery of such organisms in the fluid obtained by exploratory puncture of lymphatic glands, whether of the neck or groin. The fact that they have been recovered in this way indicates that the organisms may be found actually in those situations in certain individuals, and that their presence is accidental and not of pathological significance.

A number of the organisms studied were obtained from the intestinal contents of various animals, and were consequently derived from the material ingested by them. Those found in canned eggs may be supposed to have been introduced either after passage through fowl intestine, or by contamination with earth or by manipulation during canning. Others were found in contaminated culture tubes, etc., in the laboratory.

DIAGNOSIS TABLE.

Having dealt with the characters possessed by the different forms demarcated in the literature, and having described the cultural character of numerous examples studied in this laboratory (61 different forms have come under our notice), a few suggestions may be offered as to the best means of proceeding to a diagnosis of such organisms. The characters common to the group are—gram-positive staining,¹ spore formation, liquefaction of milk in a characteristic manner, liquefaction of gelatin,² and defective action on the sugars.³ The motility is variable, and its presence or absence is consequently of no diagnostic value as a group reaction but might

¹ There are one or two exceptions.

² Some forms liquefy gelatin exceedingly slowly.

³ By this is meant the formation of acid without gas, and the feeble extent of the former.

be useful for species-determination. First one notices the appearance on an agar slant. A 24-hour culture will present so marked a growth that it may be observed and provisionally identified till prolonged incubation has shown whether or not pigmentation develops. Such a change may not occur for several days. The agar culture presents appearances which may be grouped as follows:

DIAGNOSIS-SCHEME OF SPORE-BEARERS AND MILK-LIOUEFIERS.*

		GROWTH ON AGAR	
		{ motile	{ <i>B. subtilis</i> <i>B. robur</i>
	{ gray-white	{ dextrose fermenters	{ <i>B. ellenbachensis</i> <i>B. carotinarum</i>
		{ dextrose plus saccharose fermenters	{ — <i>B. megatherium</i>
		{ non-fermenters— <i>B. tenuis</i>	
Surface dry	{ yellowish gray	{ — <i>B. petasites</i>	
	{ chalklike	{ — <i>B. cretaceus</i>	
	{ edges feathery	{ — <i>B. mycoides</i>	{ <i>B. tumescens</i> <i>B. granulatus</i>
	{ scanty growth	{ non-saccharose fermenters	{ motile
		{ non-fermenters— <i>B. gastrophilus</i>	{ excessively motile— <i>B. asterosporus</i>
	{ minute folds	{ fatty surface— <i>B. ellenbachensis</i>	
		{ yellow white	{ feebly motile— <i>B. parvus</i>
Surface gummy	{ no folds	{ yellow— <i>B. ochraceus</i>	{ very motile — <i>B. fusiformis</i>
		{ white— <i>B. mesentericus</i>	
	{ transparent	{ milk not coagulated— <i>B. sphericus</i>	
		{ milk coagulated	{ <i>B. asterosporus</i>
Surface moist			{ <i>B. oleae</i>
	{ white	{ — <i>B. butyricus</i>	
	{ woolly edges	{ — <i>B. pumilus</i>	
		{ yellowish— <i>B. ruminatus</i>	
		{ dirty gray — <i>B. graveolens</i>	
Surface very moist and exuberant		{ white; prominent folds	{ coagulated milk— <i>B. vulgatus</i> does not coagulate milk { <i>B. atterimus</i> <i>B. geniculatus</i>
			{ non-saccharose fermenter { <i>B. silvaticus</i> <i>B. simplex</i>
		{ does not coagulate milk	{ saccharose fermenter— <i>B. mesentericus</i> sugars not affected— <i>B. fusiformis</i>
		{ coagulates milk— <i>B. pumilus</i>	
Surface glassy	{ smooth		
	{ retiform— <i>B. mesentericus ruber</i>		
	{ minute folds — <i>B. teres</i>		
	{ other-colored — <i>B. ochraceus</i> †		

* This table excludes anthrax and its relatives.

† N.B.—The pigment may not develop for several days.

In interpreting this diagnostic table, it must be constantly borne in mind that the characters are not absolutely constant in any one case. It is frequently found that the same organism will change in appearance on prolonged incubation, or, as has already been mentioned, after successive culturing or planting. Moreover,

it is difficult adequately to describe the appearances of some forms from mere lack of suitable adjectives to express them. The wrinkling of the surface, on which much stress is apparently laid, may vary considerably in form, and may be very late in appearing. But with these provisos the classification may be found useful. We have added to the existing types a few others which appeared to be new, and which were very strikingly different in aspect.

CONCLUSIONS.

1. Members of the subtilis and mesentericus group are sufficiently frequently met with as contaminators to make better acquaintance with them advisable.

2. The forms are in many cases interchangeable.

3. Certain varieties may occur in apparently normal human tissues.

4. The differential diagnosis is aided by testing the action of the particular organism upon the various sugar media, of which dextrose and saccharose are the more important.

5. The elaborate nomenclature referred to on p. 212 would be satisfactorily replaced by a classification into *B. subtilis* or *B. mesentericus*, each of which is subdivided into the following types:

A. Acidifying dextrose alone.

B. Acidifying dextrose and saccharose.

C. Acidifying dextrose, saccharose, and mannite.

D. Acidifying maltose also.

E. Having no action on sugar.

6. An organism would thus be labeled "*B. mesentericus*, type B," if it presented the ordinary features of *B. mesentericus* and presented the typical reactions on sugar.

7. The presence of variants as regards other properties would be specified when necessary.

8. The cardinal features of the group would consist in: gram-positive staining, spore formation, ability to grow at ordinary temperature, liquefaction of milk without coagulation or acid change, feeble or no pathogenicity to laboratory animals.

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PARTURIENT PARESIS (MILK FEVER) AND ECLAMPSIA.*

SIMILARITIES BETWEEN THESE TWO DISEASES.

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In June, 1909, the attention of one of us, Healy, was called by Dr. M. A. Scovell, director of the Kentucky Agricultural Experiment Station, to the condition known in veterinary medicine as parturient paresis. Dr. Scovell's intention was to have, if possible, the etiology of this condition definitely cleared up.

Owing to the press of other work, it was impossible to take up this problem until a year ago. As our studies progressed, the similarity between parturient paresis and that condition known in human medicine as eclampsia became more and more evident, and it is the object of this paper to point out the observations upon which we base this similarity.

Parturient paresis, then, according to the best veterinary authorities, "is a nervous disorder which develops suddenly in heavy milkers after calving. It is characterized by loss of sense, of consciousness, of muscular control, by hypothermia or by hyperthermia, convulsions, coma, and mellituria."¹

Eclampsia, according to the best medical authorities, "is an acute toxemia, occurring in the pregnant, parturient, or puerperal woman, and is usually characterized by clonic and tonic convulsions, loss of consciousness followed by more or less prolonged coma, and presenting characteristic lesions."²

The following are the established facts in the etiology of parturient paresis:

The most important factor is parturition, and Hess states³ that in 170 cases he did not observe it during, nor preceding labor, nor did he observe it later than 96 hours following labor.

* Received for publication February 13, 1912.

¹ Law, *Text-book of Veterinary Medicine*, 2d ed., Ithaca, N.Y., 1905.

² Williams, *Obstetrics*, New York, 1908.

³ Hess, *Schw. A.*, 1905, 47, p. 279.

Next in importance to parturition comes heavy milk production. It is a disease, not only of cows, but pre-eminently of individual cows that give the most abundant milk, and particularly at that age when they have reached their greatest productiveness. It is rare or unknown in other animals.

High feeding, high condition, drying up of the milk secretion in normally heavy milkers, and easy delivery are also important points in the etiology. Constitutional predisposition is important, and an animal which has survived one attack is more liable to another.

In the etiology of eclampsia, parturition also plays the most important rôle. Here, however, there are some slight differences. Eclampsia occurs most frequently during labor, next in frequency just before labor, and least frequently just after labor. The majority of cases occur in primipara, and twin pregnancy and hydramnios are predisposing factors. Constitutional predisposition is evident in the hereditary character insisted upon by some authors. It is also of interest to note the greater mortality of cases occurring in multipara.

Briefly, the symptoms of parturient paresis are a sudden appearance of discomfort, without evident reason, within a period of 12 to 96 hours following an easy parturition; loss of appetite; rumination ceases; the calf is neglected; the eyes are dull and clouded, with drooping eyelids, and reddened conjunctiva; the pulse normal and sometimes strong; the breathing excited; and the animal moans or groans. As the attack progresses, the senses are dulled and the legs become unsteady and soon give way, leaving the animal prostrate. She may make an effort to arise but is rarely able to do so. The head rests on the shoulder or upper flank. The somnolence increases, passing into complete torpor and insensibility; the reflexes are all abolished; the pulse becomes soft, small and almost imperceptible; the breathing slow and stertorous. There is constipation and retention of urine. Death may occur quietly from apoplexy, cerebral compression, or cardiac failure. On the other hand, death may be preceded by marked excitement, or disorderly muscular movements, amounting to convulsions, during which bones are frequently fractured and the animal otherwise seriously injured. In the beginning of the attack, the tem-

perature may rise to 103° F. or 104° F., but as the condition progresses the temperature diminishes steadily until it falls below normal. With recovery, it promptly rises again to normal. In favorable cases, after the animal is down, and even without treatment, the secretion of the milk may continue, and spontaneous defecation and micturition occur and the animal may arise and commence feeding. It is of particular interest to note that the suddenness of improvement is often as marked as that of the attack. It is also of interest to note that in some animals the hind limbs remain paralytic for days or even weeks.

While the eclamptic convulsion may occur with the suddenness of "a bolt from a clear sky," it is much more common to see the outbreak preceded by a longer or shorter period of premonitory symptoms among which occur edema, headache, epigastric pains, and disturbances of vision. The convulsion is ushered in with a fixed expression of the eyes, which soon roll from side to side. The convulsive movements appear first about the mouth, the entire face becoming distorted, then extend rapidly to the arms, body and legs. They are usually clonic but may be tonic in form. The breathing is stertorous, face congested and flushed, and the mouth foams. During the convulsions the patient is unconscious, and after the convulsions cease passes into coma. There may be but a single convulsion, after which the patient makes an uninterrupted recovery. More frequently, however, there are many convulsions, the number of which varies greatly in different cases. A fatal coma may, in rare cases, follow a single convulsion. The pulse is full and bounding, but in severe cases is weaker, more rapid, becoming soft and very small. The temperature rises at the onset to 104° F. or 105° F., and steadily falls with the improvement of the patient. The temperature may, however, remain normal. Williams calls attention to the fact that there are cases in which convulsions do not occur, and states that a correct diagnosis can usually be established only by the demonstration of typical eclamptic lesions at autopsy. He further states, that in the few instances reported in which the disorder did not appear until several weeks after the birth of the child, the cases were probably not eclamptic at all, but that the seizures were due to other causes.

The urinary findings are the most important clinical features of eclampsia; there is always diminished secretion of urine, and frequently almost entire suppression. Albumen is almost always present, but this albuminuria is only temporary and rapidly disappears. The total nitrogen is diminished, as is also the urea, and the ammonia is markedly increased. Hyalin, granular and a few epithelial casts are found microscopically, as well as isolated renal cells and blood cells.

In parturient paresis, the following urinalyses of three cases will show that the urinary findings also constitute the most important clinical features. There is always diminished secretion, as well as retention of urine, the retention in one of our cases lasting 22 hours. There is always albumen present. The total nitrogen is increased. The hippuric acid is diminished, while the urea is increased. The ammonia is markedly increased. Microscopically, one finds hyalin, granular and a few epithelial casts and leukocytes.

As the result of 100 urinalyses of the dairy cow, covering a period of three winter months and three summer months, we have established the following as a normal clinical urinalysis:

Physical properties.—Colorless to deep yellow, clear, either no precipitate or a slight flocculent, or granular and occasionally a heavy white precipitate of calcium sulphate or triple phosphates. Reaction, alkaline, neutral or amphoteric, specific gravity—1.014.

Chemical properties.—Hippuric acid, 1.17 per cent; urea, 1.06 per cent; ammonia, 0.0009 per cent; total nitrogen, 0.58 per cent; no albumen; no sugar.

Microscopical examination.—Crystals of calcium sulphate, calcium oxalate, triple phosphates, ammonium urate, amorphous urate, squamous epithelial cells and irregular vegetable cells.

The following are the urinalyses of three cases of parturient paresis:

1.—Seven years, fifth calf, first attack, attack occurred 12 hours after labor. Her recovery was prompt and complete, with the oxygen treatment. Her mother died of parturient paresis.

Urinalysis.—Deep yellow, clear, white flocculent precipitate, strongly alkaline, specific gravity, 1.055; hippuric acid, 0.93 per cent; urea 3.23 per cent; ammonia, trace; total nitrogen, 1.58 per cent; abundant albumen; sugar, 6.25 per cent.

Microscopical examination.—Hyalin, granular and epithelial casts; quantities of leukocytes, squamous epithelial cells and irregular vegetable cells.

2.—Six years, fourth calf, first attack; attack occurred six hours after labor. Prompt and complete recovery, with the oxygen treatment.

Urinalysis.—Yellow, slightly cloudy, flocculent precipitate, amphoteric; specific gravity, 1.023; hippuric acid, 0.34 per cent; urea, 2.2 per cent; ammonia, 0.037 per cent; total nitrogen, 1.05 per cent; abundant albumen; sugar, 2.2 per cent.

Microscopical examination.—Numerous small granular casts, some large granular casts, and a few hyalin casts; quantities of degenerating squamous and cuboidal epithelial cells, leukocytes and irregular vegetable cells.

3.—Seven years, fifth calf, first attack, attack occurred 36 hours after labor. Recovery prompt and complete, with the oxygen treatment.

Urinalysis.—Yellow, clear, slight flocculent precipitate, slightly acid; specific gravity, 1.018; hippuric acid, 0.83 per cent; urea, 1.20 per cent; ammonia, 0.035 per cent; total nitrogen, 0.65 per cent; moderate amount of albumen present, sugar 1.64 per cent.

Microscopical examination.—Granular and hyalin casts, squamous epithelia and leukocytes, irregular vegetable cells.

The characteristic lesions found at autopsy in cases of eclampsia are areas of necrosis, in which blood cells may or may not be present in the liver, in the neighborhood of the smaller portal vessels; that is a hemorrhagic hepatitis. There is an acute parenchymatous degeneration of the kidneys, with but little disturbance of the glomerule and interstitial substances. There is also a coagulation necrosis, either focal or diffuse, of the adrenals, and pneumonia is not infrequent.¹

Unfortunately, the finer pathological changes occurring in parturient paresis have not been established, and none of the three cases which we had during the past year died. Nevertheless, we shall show in our second paper, that three guinea-pigs which died

¹ Adami and Nichols, *Principles of Pathology*, 2, 1911. (This contains the best description of the kidney of pregnancy which we have seen.)

under the influence of the toxin of parturient paresis in five, six, and seven days, presented upon microscopical examination, areas of hemorrhagic necrosis in the liver, an acute parenchymatous nephritis with interstitial hemorrhage, and an acute degeneration of the adrenals with interstitial hemorrhage; and, in one case, a left lobar pneumonia. That in another guinea-pig, which received a smaller amount of the toxin, the immediate result was an intense diuresis, accompanied with an albuminuria, the animal passing more than 200 c.c. of urine in the 24 hours. This animal apparently recovered, and 10 days later was chloroformed. Upon microscopical examination, we found an acute parenchymatous nephritis, with some interstitial hemorrhage; a rather extensive necrosis of the liver cells but without hemorrhage; and localized areas of necrosis in the adrenals.

We now come to the most interesting part of this whole problem, namely, the cause underlying, on the one hand, eclampsia, and on the other, parturient paresis.

As Zweifel has aptly put it, "Eclampsia is the disease of theories." It has been identified with uremia; it has been held that it was the result of anemia and edema of the brain; of the circulation of ammonium carbonate in the blood; that it was the result of bacterial invasion; of the circulation of carbamic acid in the blood; that it was due to poisons arising from the fetal elements; that it was due to poisons absorbed from the placenta; that it was due to thrombosis, caused by placental cells passing into the maternal circulation; that it was due to the circulation of lactic acid in the blood; that it was due to thyroid insufficiency; and again to irregularities in the metabolism of the parathyroid bodies; and, lastly, that it was due to autointoxication. There is not sufficient experimental evidence, however, to establish any of the above theories, and it is of interest to note that the possibility of the breast elaborating the toxin has not, as yet, been investigated.

Parturient paresis has also been explained by many theories. It was considered the result of emotional excitement; of cerebral anemia; of cardiac hypertrophy; of bacterial infection; as the reaction of surplus nerve force, following an easy labor; and of an increased percentage of sugar in the blood. The most remarkable

feature presented by parturient paresis is the rapid response to, and the entire success of, the modern treatment. This treatment has reduced the mortality from 60 per cent to 0.5 per cent, and the rapidity of recovery is very striking indeed. An animal, apparently in the last stage of coma, will often within a few hours, or even within twenty minutes, after the treatment, be up and eating and licking her calf, apparently well and contented. The treatment consists of acute dilatation of the udder, by means of suitable liquids or gases. The treatment was first introduced by J. Schmidt of Kolding, Denmark, and was based upon the theory that the disease was due to a bacterial infection of the udder. He used for injections into the udder solutions of potassium iodid, but it has since been shown that solutions of lysol, or of sodium chlorid, will act as well, and that the injections of sterile water, sterile air, and of oxygen are even more successful. Injections of oxygen or of sterile air now constitute the generally used treatment.

That the disease is due to a toxin elaborated in the udder, as the result of its own metabolism preceding normal milk production, there can be no reasonable doubt, and that the success of the modern treatment is due to preventing, by means of pressure, the absorption of this toxin seems most highly probable.

We are of the opinion that eclampsia is due to a similar toxin elaborated by the breast in a similar manner, and would strongly recommend, as the most promising treatment, dilatation of the breasts with oxygen or sterile air, accompanied by vigorous massage of the breasts, or forcible compression of them by means of a properly applied bandage, at the same time using whatever medical measures may be indicated. We ourselves shall thoroughly test this method of treatment as soon as the opportunity occurs.

THE TOXIC CHARACTER OF THE COLOSTRUM IN PARTURIENT PARESIS.*

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In our first article on the resemblances between parturient paresis and eclampsia we have given the essential characteristics of both of these diseases. There is, therefore, no need to go into this subject farther, except to say that parturient paresis is a disease which among the lower animals seems to be confined to plethoric cows and heavy milkers. As pointed out by Law,¹ it is essentially a disease of the cow by reason of the fact that, of all domestic animals, the cow alone has been bred long and systematically for the purpose of securing the greatest powers of digestion and assimilation and the highest yield of milk. In other words, as the result of careful breeding the cow has been converted into a living machine for the transformation of various food-stuffs into milk. Parturient paresis is pre-eminently a disease of plethoric, heavy-milking breeds of cows and of those individual animals which give the greatest yield of milk. The disease is rare or unknown in the scrub cow or among common herds, and it is fatal in the best milking breeds. It is influenced by age, feeding, ease of calving, drying up of the milk secretion before calving, parturition, such weather conditions as influence the character and abundance of the food supply, and by certain individual idiosyncrasies and constitutional predispositions, by cardiac hypertrophy and by increased blood tension in the arteries and capillaries and by the driving of the blood from the great vascular viscera back of the diaphragm, as the result of severe compression, in the expulsive movements of labor. Emotional excitement must be practically excluded as a cause of this disease. Among the prime and immediate causes we have parturition, a permanent or transient plethoric condition of

* Received for publication February 13, 1912.

¹ *Text-book of Veterinary Medicine*, 2d ed., 3, pp. 301-17. Ithaca, N.Y., 1905. This book contains the best account of parturient paresis that we have thus far seen.

the blood vessels, with corresponding increase of pressure on the nerve centers of the brain, the phenomenal trophic and secreting activity of the udder of heavy milkers and the intensely active physiological activity of the mammary glands, resulting in the sudden rise and absorption into the circulation of leukomaines or toxic alkaloids of the cells of the mammae. These, according to Law, are the principal causes operating to bring on an attack of this disease. In the present state of our knowledge it is of little moment whether we call the substances other than milk resulting from the sudden disintegrative changes occurring in the udder at, or about the time of parturition, leukomaines, alkaloids, toxins, or what not. It seems reasonably certain, however, that no gland of the size and physiological activity of the udder of a heavy milking cow but must contribute largely and sometimes malignantly to the internal secretions of the animal. The question, therefore, immediately before us in the study of this acutely toxic condition in the cow, is to determine experimentally whether immediately preceding or during an attack of this disease the udder does actually secrete poisonous substances which if not quickly eliminated or prevented from entering the blood-current could be held responsible for this disease. It therefore occurred to one of us, Kastle, to test the conduct of the first colostrum of the cow, obtained during an attack of parturient paresis, upon the lower animals.¹ Accordingly these tests have been carried out on the guinea-pig.

Our experiments have also included a few other substances besides the colostrum of cows suffering from parturient paresis, such as the first colostrum of the normal cow, fresh milk from the Station herd, the urine of the normal cow and the first urine of a cow suffering from parturient paresis, normal salt solution, (0.85 per cent NaCl), and the aqueous solution of certain residues from colostrum and milk left after precipitating the colostrum and milk

¹ On the day after our three papers on parturient paresis and eclampsia were mailed to the editor of the *Journal of Infectious Diseases*, Chicago, viz, on February 13, 1912, Dr. Surface called our attention to an abstract by Helfer of a paper by Hoyois in the *Berliner Tierärztliche Wochenschrift*, October 5, 1911, No. 40, pp. 727-28, the original of which appeared in the *Annales de Médecine vétérinaire de Bruxelles*, July-Aug.-Sept., 1910?, in which according to Hoyois the colostrum in cases of parturient paresis, on intraperitoneal injection in doses of 10 to 20 gms., caused paralyzing symptoms in rabbits and guinea-pigs, with subsequent death at the end of seven to 12 days.

with dilute acid, and evaporating. In the following we give the results of these experiments under their respective headings as to the substances tested.

FRESH MILK.

Experiment 1.—A healthy male guinea-pig received into the peritoneal cavity by hypodermic injection, 5 c.c. of fresh milk containing 22,500 bacteria per c.c. This pig seemed distressed for an hour or so, and then ate a little. On the following morning he had some diarrhea, from which he promptly recovered. One month later he was alive and well.

Experiment 2.—A healthy male guinea-pig received into the peritoneal cavity, by hypodermic injection, the dried residue of the filtrate of 200 c.c. of fresh milk, which had been precipitated with alcohol. The dried residue was rubbed up with 10 c.c. of sterile distilled water before injecting. This pig showed no discomfort, had no diarrhea. One month later he was alive and well.

COLOSTRUM FROM NORMAL COW.

Experiment 3.—A healthy male guinea-pig received into the peritoneal cavity, by hypodermic injection, 5 c.c. of fresh, first colostrum from a normal cow. This colostrum contained 3,000 bacteria per c.c. mostly streptococci. This pig seemed distinctly distressed for remainder of day. The following morning he had a bad diarrhea from which he promptly recovered. One month later he was alive and well.

Experiment 4.—A healthy male guinea-pig received into the peritoneal cavity by hypodermic injection, 5 c.c. of fresh colostrum from a normal cow which had calved 60 hours previously. The cow had induration of one quarter of the udder. No bacterial count was made of this colostrum. This pig seemed distressed for an hour or so, and then ate some. The following morning he had some diarrhea from which he promptly recovered. One month later he was alive and well.

Experiment 5.—A healthy, male guinea-pig received into the peritoneal cavity, by hypodermic injection, the dried residue of the filtrate of 100 c.c. of fresh first colostrum prepared as in Experiment 2. This pig showed no discomfort and had no diarrhea. One month later he was alive and well.

Experiment 6.—A healthy, male guinea-pig received into the peritoneal cavity, by hypodermic injection, the dried residue from 100 c.c. of fresh colostrum from a cow which had calved 60 hours before colostrum was obtained. The material was prepared as in Experiment 2. This pig showed no discomfort, the following morning he had a diarrhea from which he promptly recovered. One month later he was alive and well.

Experiment 7.—A healthy guinea-pig (sex not observed) received into the abdominal cavity, by hypodermic injection, 3 c.c. of the fresh, first colostrum from a normal cow, which had been diluted one in three with water. This pig showed no discomfort, two days later had a slight diarrhea. It now received in a similar manner 5 c.c. of the colostrum diluted with equal volume of water. The following morning had diarrhea which continued several days. One month later the pig was alive and well.

Experiment 8.—A healthy guinea-pig (sex not observed) received into the abdominal cavity, by hypodermic injection, 2.5 c.c. of colostrum whey which had

been obtained by precipitating the colostrum with acetic acid, filtering and neutralizing the filtrate with N/10 sodium hydroxid and diluting with four times its volume of water. This pig showed no discomfort, had no diarrhea. Two days later received 5 c.c. of colostrum whey diluted with equal volume of water and prepared as above. The pig showed no discomfort, had no diarrhea. One month later he was alive and well.

COLOSTRUM FROM COW ILL WITH PARTURIENT PARESIS.

*Experiment 9.*¹—A healthy, female guinea-pig received, in the manner above indicated, 10 c.c. of fresh, first colostrum from a cow ill with parturient paresis. The colostrum contained 34,600 bacteria per c.c. This pig seemed well for six days, had no diarrhea at any time, and then died. On post-mortem examination this pig showed acute parenchymatous nephritis with interstitial hemorrhages, acute parenchymatous hepatitis with interstitial hemorrhages, acute degeneration of the cells of the adrenal

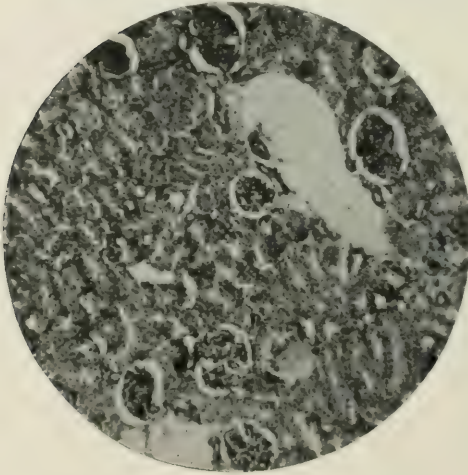


FIG. 1.—Experiment 9. Acute Nephritis. $\times 100$.

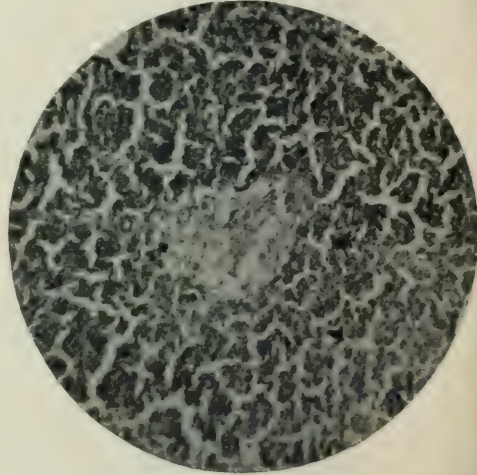


FIG. 2.—Experiment 9. Liver: Focal Necrosis. $\times 100$.

cortex, with complete destruction of the medullary cells, and interstitial hemorrhages. (Fig. 1, 2, 3.) There was no evidence of tuberculosis. The pig was not pregnant. Cultures from the liver, kidney, and spleen were negative. No peritonitis.

Experiment 10.—A healthy, female guinea-pig received as above indicated 10 c.c. of the fresh, first skimmed colostrum from a cow ill with parturient paresis. This pig seemed well and had no diarrhea for five days and then died. On post-mortem this pig showed acute parenchymatous nephritis, with interstitial hemorrhage; acute parenchymatous hepatitis with interstitial hemorrhage, and marked peripheral necrosis; acute degeneration of cells of the adrenal cortex, with complete destruction of the medullary cells, and interstitial hemorrhages. (Fig. 4, 5, 6, 7.) There was no evidence of tuberculosis. The pig was in the very early stages of pregnancy. Cultures from the liver, kidney and spleen were negative. No peritonitis.

¹ In our abstract of this paper published in the *Proceedings for the Society of Experimental Biology and Medicine*, pigs 9, 10, and 11 are referred to by the numbers 1, 2, and 3, respectively.

Experiment 11.—A healthy, female guinea pig received in the manner above indicated 10 c.c. of fresh, first colostrum cream of a cow ill with parturient paresis. This pig seemed well and had no diarrhea for six days, and then died. Aborted during

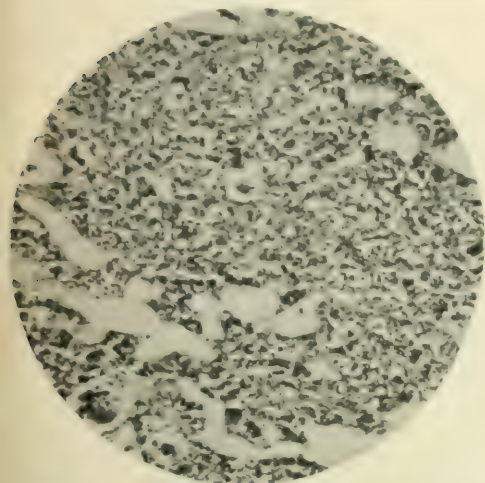


FIG. 3.—Experiment 9. Adrenal: Necrosis of Medulla. $\times 100$.

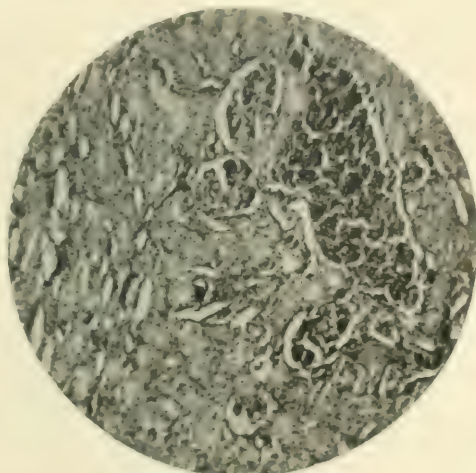


FIG. 4.—Experiment 10. Acute Nephritis: Hemorrhage. $\times 100$.

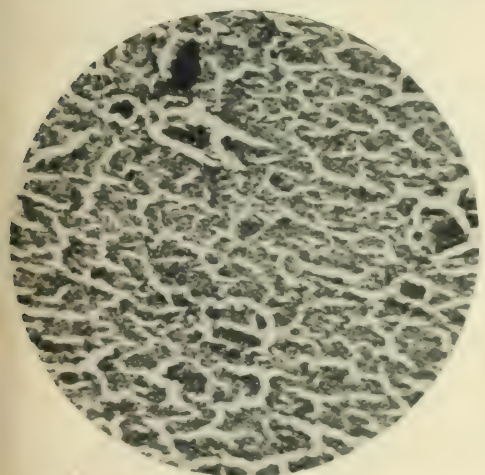


FIG. 5.—Experiment 10. Liver: Hepatitis; Hemorrhage. $\times 100$.

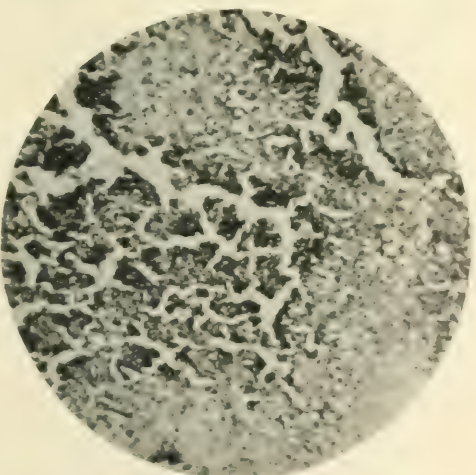


FIG. 6.—Experiment 10. Adrenal Cortex: Hemorrhage. $\times 100$.

the first 12 hours. Fetus 5.5 cm. long. On post-mortem this pig showed acute parenchymatous nephritis, with interstitial hemorrhages, acute parenchymatous hepatitis, with areas of complete necrosis, and interstitial hemorrhages. Some degeneration of the cells of the adrenal cortex, with complete destruction of medullary

portion and interstitial hemorrhages. (Fig. 8, 9, 10, 11.) Acute lobar pneumonia of left lung. There was no evidence of tuberculosis. Cultures from liver, kidney and spleen negative. Culture from lung contained a diplococcus; no peritonitis except over upper and anterior surfaces of liver.

Experiment 12.—A healthy, female guinea-pig received, as above indicated, 10 c.c. of skimmed colostrum of a cow ill with parturient paresis, which had been kept 17 days at 8° C. The colostrum was very acid, reaction equal to 10.5 per cent normal sodium hydroxid. This pig steadily lost weight, otherwise seemed well, had no diarrhea and lived 16 days, then died.

Experiment 13.—A healthy, female guinea-pig received, as above indicated, 10 c.c. of the 17-day old colostrum, which had been neutralized with normal sodium

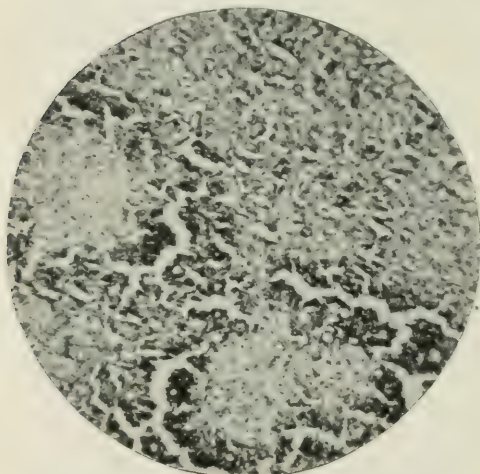


FIG. 7.—Experiment 10. Adrenal Cortex: Necrosis; Hemorrhage. $\times 100$.

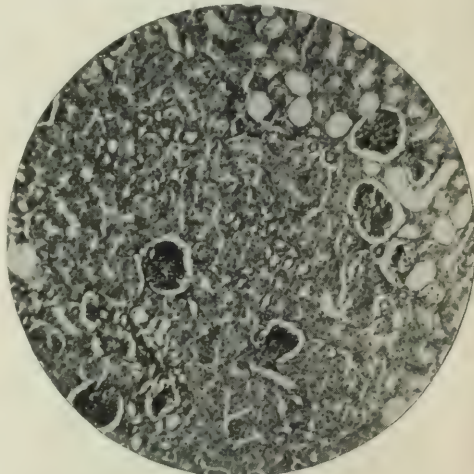


FIG. 8.—Experiment 11. Acute Nephritis. $\times 100$.

hydroxid. The colostrum was from a cow ill with parturient paresis. This pig steadily lost weight, has not seemed quite well at any time. Had no diarrhea. Aborted on the fifth day. Fetus was 8 cm. long. This pig is still living, and is regaining weight. There is a slight increase in the quantity of urine which contains albumen.

Experiment 14.—A healthy, female pig received, as above indicated, 10 c.c. of 17-day old colostrum which had been neutralized with normal sodium hydroxid and boiled. Colostrum was from a cow ill with parturient paresis. This pig steadily lost weight, had no diarrhea, aborted within the first 12 hours. Fetus 8 cm. long. This pig is still alive, and is regaining weight. There is a slight increase in the quantity of urine which contains albumen.

NORMAL SALT SOLUTION ON PREGNANT GUINEA-PIGS.

Experiment 15.—A healthy, female guinea-pig, five to seven weeks pregnant, received into the abdominal cavity, by hypodermic injection, 10 c.c. of sterile, normal salt solution (0.85 per cent NaCl) at 38° C. This pig showed no discomfort and had not aborted at the end of five days. Had no diarrhea. At the end of five days from

the time the pig had received the injection of normal salt solution she received intraperitoneally by injection 10 c.c. of the fresh milk from the Station herd, which had previously been heated to 38° C. This caused no discomfort and no diarrhea. She

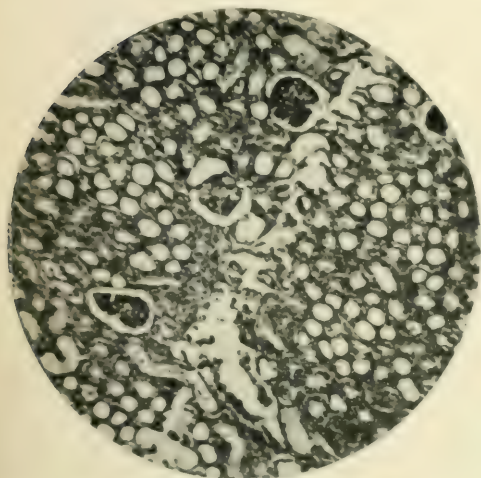


FIG. 9.—Experiment 11. Nephritis: Destruction of epithelial cells. $\times 100$.

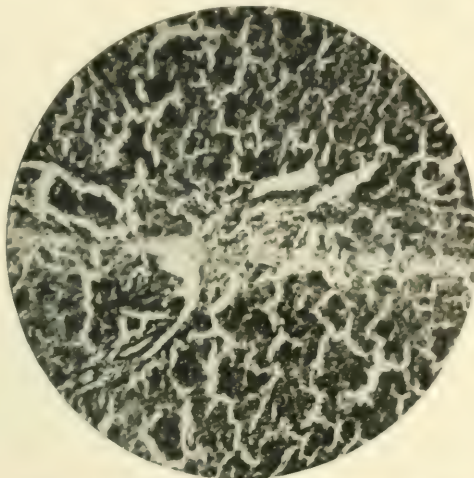


FIG. 10.—Experiment 11. Liver: Hepatitis: Hemorrhage. $\times 100$.

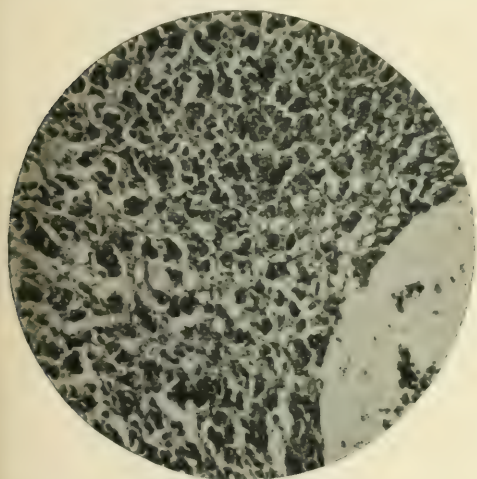


FIG. 11.—Experiment 11. Adrenal: Necrosis of Cortex and absence of Medulla. $\times 100$.

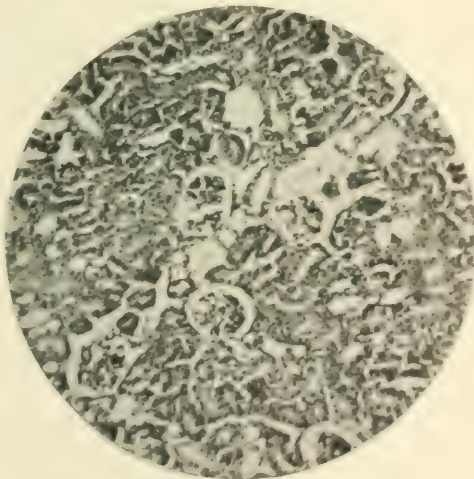


FIG. 12.—Experiment 18. Acute Nephritis. $\times 100$.

had not aborted at the end of four days. At the end of this time she received by intraperitoneal injection 8 c.c. of the first, fresh, whole colostrum of a normal cow (second calf), which had previously been heated to 38° C. Following this injection,

this pig aborted in 60 hours, giving premature birth to two fetuses, each 6.5 cm. in length, and 60 hours after this she aborted a second time, giving premature birth to one fetus, 7 cm. in length.

FRESH MILK FROM STATION HERD ON PREGNANT GUINEA-PIG.

Experiment 16.—A healthy, pregnant guinea-pig received, by intraperitoneal injection, 10 c.c. of fresh milk from Station herd, the milk being previously heated to 38° C. before the injection. This pig suffered some discomfort from the injection but had not aborted at the end of five days. She was not well, however, showing loss of appetite and falling off in weight. At the end of five days the pig was chloroformed. The post-mortem showed accumulation of blood in left side of peritoneal cavity, and some unabsorbed particles of cream over omentum. Pregnant in left horn of uterus,

two fetuses, 1 cm. in length. Right horn of uterus empty, and evidently had not been pregnant. The bad condition of this pig was undoubtedly due to puncture of a blood-vessel in the peritoneal cavity, as a result of the injection.

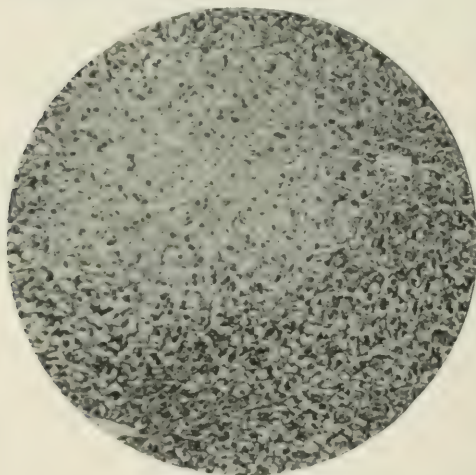


FIG. 13.—Experiment 18. Liver: Necrosis. $\times 100$.

COW'S URINE.

Experiment 17.—A healthy, male guinea-pig received, as above indicated, 10 c.c. of fresh clear urine of normal cow. This pig showed no discomfort, had no diarrhea, and no diuresis. Lived seven days and was then chloroformed. On post-mortem, organs and tissues were found normal.

Experiment 18.—A healthy, male guinea-pig received, as above indicated, 10 c.c. of the first, fresh, clear urine of a cow ill with parturient paresis. This pig showed no discomfort, and no diarrhea, but had a very marked diuresis, passing at least 200 c.c. urine in 24 hours. This urine contained a small amount of albumen and no sugar. This pig recovered from the diuresis and seemed well and was chloroformed on the thirteenth day. The post-mortem showed small areas of acute parenchymatous nephritis, without interstitial hemorrhages, rather extensive necrosis of the liver cells, especially in the periphery of the lobules, but without interstitial hemorrhages. Localized areas of necrosis in the adrenal cortex, with normal medullary layer. (Fig. 12, 13.)

It is evident from these results that normal salt solution, fresh milk from normal cows, the colostrum of normal cows, and the urine of a healthy cow cause no serious disturbances in the normal guinea-pig, when injected into the peritoneal cavity. The injection

of the colostrum of normal cows invariably caused a diarrhea in healthy guinea-pigs, male and female, from which they gradually recovered after a few days. In this connection it is interesting to note that it has long been known that human colostrum acts as a mild cathartic on the suckling.¹ As already indicated in the above (see Experiment 16), the pregnant guinea-pig that received the injection of the milk of a healthy dairy herd did experience a good deal of discomfort immediately following the injection. This was followed by loss of appetite and corresponding loss of weight, during the four days that the pig lived. On January 29 this pig was chloroformed. The post-mortem showed a rupture of one of the blood vessels in the peritoneal cavity, and in all probability the abnormal condition of this pig after the injection of the fresh milk was the result of slow bleeding into the body cavity. No evidence was obtained to show that the pig had aborted. With this possible exception which evidently resulted from accidental, mechanical injury, and with the exception of the diarrhea resulting from the injection of normal colostrum, no noteworthy disturbances followed from the intraperitoneal injection of normal salt solution, fresh milk, normal colostrum, or the urine of the normal cow. On the other hand, death resulted from the injection of the colostrum of the cow having parturient paresis, and the post-mortem and microscopical examinations of the organs of the four guinea-pigs that were thus killed showed the same pathological degenerations and changes that are characteristic of eclampsia. Unfortunately but little if anything seems to be known regarding the micropathology of parturient paresis in the cow. We have shown, however, that cows recovering from an attack of this disease invariably show a nephritis. Our results with the colostrum of the cow suffering with parturient paresis certainly go to show the presence therein of some substance toxic to guinea-pigs and certainly point to the udder and the mammary glands as the place of origin of the toxins or internal secretions producing parturient paresis and eclampsia respectively. The fact that the urine of the cow ill with parturient paresis causes such a profound diuresis in the guinea-pig also points to the presence of toxic substances in the urine of the animals so affected and

¹ Williams, *Obstetrics*, New York, 1908, pp. 351-52.

indicates that these toxins are not entirely destroyed in the tissues of the cow, but are in part at least, and it may be in somewhat modified form, excreted by the kidneys.

The fact also that the colostrum of the cow during an attack of parturient paresis invariably caused an abortion in pregnant guinea-pigs is a matter of considerable physiological significance and will be discussed more fully in the following paper.

We hope in the near future to attempt the isolation of the particular substance in the colostrum or the udder responsible for parturient paresis, or, at any rate, its more careful study and accurate identification, and also to undertake a careful microscopic and chemical study of the mammary gland in this condition. Obviously this must be deferred until we can obtain the material necessary for this investigation.

The idea that parturient paresis is the result of some toxic internal secretion product elaborated in the udder of the cow, its absorption into the blood and action on the nerve centers, is in harmony with all that is known concerning the etiology of this disease and with the modern therapeutic practices which have proven of such inestimable benefit in its treatment and cure. In this connection it is only necessary to point that these several modes of treatment have effected a reduction in the mortality of this disease from 70 to 15 and finally to less than 1 per cent, and can be most readily explained on the assumption that through extreme dilatation of the udder by oxygen or sterile air, the blood supply of the mammary gland is practically cut off entirely or at any rate greatly diminished, until the milk gland has the opportunity to resume its ordinary excretory activity, thereby eliminating the toxic products in the colostrum and the milk.

In 1897 Schmidt effected a great reduction in the mortality from this disease by the use of sterile solutions of potassium iodid. Later aqueous solutions of other antiseptic substances were found equally efficacious and still later it was found that normal salt solutions accomplished the same result. Obviously no antiseptic or antidotal properties can be described to an 0.85 per cent solution of common salt. In this connection the practice of the Jersey Island dairymen is of great interest. This consists merely in leaving

the udder un milked for 24 hours after calving. In this way their predisposed cows were protected against an attack of parturient paresis. Obviously in such cases the necessary dilatation of the udder was effected through its own secretions. Finally there came into modern veterinary practice the use of oxygen gas and later the use of sterilized air, whereby the mortality from this disease was reduced to less than 1 per cent. Here the dilatation necessary to the prevention of the absorption of poisonous products is produced by a comparatively inactive mixture of gases, its only other possible effect being to maintain an aerobic condition of the udder, until the gland has had the opportunity to resume its normal excretory functions. Upon no other grounds than those involving the elaboration within the udder, at the period of parturition, of toxic substances and the passage of these into the blood stream, do we have so complete an understanding of the saving of animal life by the therapeutic methods now employed in the treatment of parturient paresis. While by no means an uncommon pathological condition among plethoric, heavy-milking dairy cows, it is not always an easy matter to secure a case of this disease at the moment when it is desired. We therefore reserve the right to continue these investigations along the lines indicated in the above, with the object of throwing further light on the nature of the toxin contained in the colostrum of cows suffering with parturient paresis, and the possible occurrence of this toxin in the colostrum of women suffering from eclampsia, and with the still further object of studying the precise conditions under which it is elaborated in the udder and mammary glands.

THE INTERNAL SECRETION OF THE MAMMAE AS A FACTOR IN THE ONSET OF LABOR.*

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(From the Laboratory of the Kentucky Agricultural Experiment Station, Lexington, Kentucky.)

The importance of the internal secretions has come to be well recognized in modern physiology and certain of these secretions, notably that of the thyroid and suprarenals, have been turned to great practical account in modern therapy. The profound influence of one organ or gland upon another organ or gland, through the stimulus of its peculiar internal secretion, has also been brought to light through the masterly researches of Bayliss and Starling.¹ The rapid secretion of the pancreatic fluid under the influence of secretin is a sight that will never be forgotten when witnessed for the first time, and when one recalls the former difficulty of getting even a few cubic centimeters of the pancreatic secretion. It would lead us too far afield from the subject of the present communication even to mention the various hormones which have been discovered since Bayliss and Starling's first epoch-making research upon secretin, not to mention the various applications of the hormone theory to biochemical phenomena that have been made in the past few years.² Among these may be mentioned the fact, however, that Miss Lane-Clayton and Starling³ have shown that the stimulus to the hypertrophy and lacteal activity of the mammary gland in pregnant animals comes not from the ovaries, or placenta, or uterus, but from the fetus itself. These observers found that injections of aqueous extracts of rabbit fetuses into a virgin rabbit every one to three days over a period of three weeks, led the glands, which were at first almost invisible, to become markedly hypertrophied, with enlargement of the ducts and epi-

* Received for publication February 13, 1912.

¹ The Croonian Lectures, *Lancet*, 1905, 1, 2.

² Armstrong, H. E., and Armstrong, E. F., *Proc. Roy. Soc.*, 1910, S.B., 82, p. 588; *Jour. Lond. Chem. Soc.*, 1910, Abs. II, p. 883.

³ *Proc. Roy. Soc.*, 1905-6, S.B., 77, p. 505. See also for a general discussion of the hormones, Howell, *Science*, 1910, 31, p. 93.

thelium, and to the discharge of a thin fluid and in multiparous rabbits to the discharge of true milk. At present nothing apparently is known concerning the stimulus which brings on the onset of labor in the pregnant female, and nothing as to the cause of premature or delayed labor. It is known of course that this can be accomplished in certain instances by the action of certain drugs, such as ergot; or by mechanical injury to the fetus, or by shock or emotional disturbances or even perhaps by pronounced fatigue, but as yet nothing is known concerning the precise causes which bring about normal labor in the pregnant female.

Our attention was first directed to this subject as the result of our studies on the effect of the colostrum of a cow, ill with parturient paresis, on guinea-pigs. It will be seen from Experiment 11, the details of which are given in our second paper, that a pregnant guinea-pig aborted during the first 12 hours following the intraperitoneal injection of 10 c.c. of fresh, first colostrum cream of a cow ill with parturient paresis. Fetus 5.5 cm. in length.

In another case, Experiment 13 (second paper), a pregnant guinea-pig aborted on the fifth day after receiving intraperitoneally 10 c.c. of the colostrum of the cow, ill with parturient paresis, and which had been kept in the refrigerator for 17 days, and neutralized with sodium hydroxid immediately before the injection. Fetus 8 cm. in length.

The unexpected results of these experiments, in so far as the abortion is concerned, naturally led us to believe that the colostrum of the cow, ill with parturient paresis, contains a substance, or substances, capable of stimulating the mother to premature labor; and to the further thought that perhaps this substance, or substances, is not confined to cows suffering from parturient paresis, but is present also in the colostrum of normal cows or for that matter in that of healthy animals generally. For fear that the absorption had been brought about in Experiments 11 and 13 (second paper) by some imperceptible mechanical injury or by the large volume of liquid injected, we have controlled these experiments, on pregnant guinea-pigs, by the injection of normal salt solution and fresh milk from a healthy dairy herd. In this connection the results of Experiment 15 (second paper) are of particular interest.

It will be observed that a pig in the fifth to seventh week of pregnancy did not abort in five days following an injection, intraperitoneally, of 10 c.c. sterile, normal salt solution (0.85 per cent NaCl). Five days after she had received the injection of the normal salt solution, she received by the intraperitoneal injection, 10 c.c. of fresh milk from a healthy dairy herd. This caused no apparent discomfort and no abortion after four days. She now received, by intraperitoneal injection, 8 c.c. of the first, fresh, whole colostrum of a normal cow (second calf), which had been heated to 38° C. immediately before the injection. Following this last injection this pig aborted in 60 hours, giving premature birth to two fetuses, each 6.5 cm. in length, and 60 hours after this she aborted a second time, giving premature birth to one fetus, 7 cm. in length.

That the normal colostrum of the cow contains a substance capable of causing abortion in pregnant guinea-pigs is also shown by the results of Experiments 19 and 20.

Experiment 19.—A healthy, female guinea-pig five to seven weeks pregnant received by intraperitoneal injection 8 c.c. of skimmed, boiled colostrum from a normal cow. This colostrum was cooled to 38 C. before the injection. The pig showed no discomfort and ate cabbage one hour after the injection. Eight days later she aborted, giving premature birth to two fetuses, one 7 cm. long and weighing 17.7 gms., and the other 7.5 cm. long, and weighing 26.7 gms.

Experiment 20.—A healthy, female guinea-pig, five to seven weeks pregnant, received by intraperitoneal injection 8 c.c. of the whole, fresh normal colostrum. The pig showed no discomfort and ate cabbage one hour after the injection. Had no diarrhea and at the end of eight days aborted two fetuses, one of which was 9.5 cm. long and weighed 47.7 gms., the other was about the same size and age. Both were covered with hair.

We have still another case to show that fresh milk from a healthy dairy herd and certain forms of mechanical injury are incapable of bringing about abortion in pregnant guinea-pigs. That such is the case is seen from Experiment 16 (second paper). In this experiment it is evident that the intraperitoneal injection of 10 c.c. of fresh milk does not produce abortion in pregnant guinea-pigs.

Neither did the rupture of a blood-vessel in the peritoneal cavity, with considerable internal hemorrhage as the result of mechanical injury during the injection.

It is evident from these results that the colostrum of the normal cow, as well as that of the cow suffering from parturient paresis, contains a substance, or substances, capable of bringing about abortion in pregnant guinea-pigs.

Whether this substance causing the abortion is a hormone or a toxin can only be determined by further experiment, which we hope to undertake as soon as the necessary material can be obtained.

It will be seen from Experiment 19, however, that the substance, or substances, in the fresh colostrum of the normal cow which excite the pregnant guinea-pigs to premature labor, withstands heating to boiling for a short time. In this respect it is similar to the hormones and differs from the soluble ferments and many toxins. At any rate, we have evidence here of a new and hitherto unrecognized correlation between the mammary glands and the uterus. According to Lane-Clayton and Starling the fetus through its internal secretions stimulates the hypertrophy and lacteal activity of the mammary gland. It is evident from our experiments that the internal secretions of the mammary gland stimulate the mother to labor and the birth of the offspring.

In this connection it is of interest to note that only the mammalia carry their young, and therefore have labor.

We hope to continue these experiments with the view of learning something as to the precise nature of the substance capable of causing abortion in pregnant guinea-pigs and of determining whether the colostrum of other species of animals, besides the cow, has the power of stimulating the pregnant animal to premature labor.

In conclusion we desire to express our thanks to Mr. J. W. Nutter, assistant in dairying, for much valuable assistance in the practical details of these investigations, and to Professor L. E. Nollau, assistant professor of drawing, for making the photomicrographs used in the illustration of these papers.

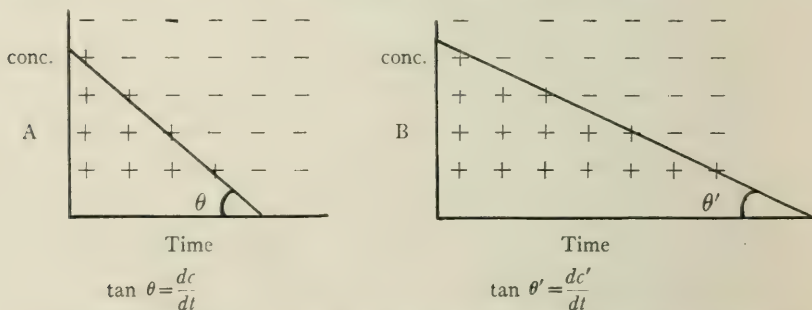
SOME REMARKS ON THE RIDEAL-WALKER TEST AND ON THE RIDEAL-WALKER METHOD. WITH SPECIAL REFERENCE TO THE "LIFE FACTOR" AND TO THE "MECHANICS OF DISINFECTION" AND THEIR INFLUENCE ON VELOCITY AND EQUILIBRIUM VALUES.*

S. RIDEAL AND E. K. RIDEAL.

Within the last year or so several attempts have been made to measure the rate of disinfection, and incidentally several attacks have been made on the Rideal-Walker test. These can have arisen only through an insufficient appreciation of the Rideal-Walker method and what is claimed for it.

The Rideal-Walker test is a measure of the total possible germicidal work a disinfectant can do in terms of an arbitrary standard whose absolute value is unknown. This work is done under fixed conditions of temperature and time on a fixed working substance. (Whether this fixed working substance is homogeneous or not will be discussed later.)

No account, however, is taken of the velocity, although velocity measurements are easily obtained from the slope of the curves obtained in the Rideal-Walker method. Thus with the two disinfectants A and B in the diagrams, the velocities are:



This problem of rate the Rideal-Walker test apart from the method does not attempt to deal with for the following reasons:

* Received for publication February 2, 1912.

First, it is only in very rare cases that the time of partial sterilization is required, complete disinfection being generally desired, and second, although the integral death time is measurable, the fractional time or the rate of death is special for each definite set of circumstances and indeed for each experiment, as we shall endeavor to point out later. Those authors who have criticized the Rideal-Walker test from theories they have developed about rate measurements have attacked a method which is not intended to measure rates.

It is obvious that in order to determine a disinfectant velocity we have to measure (1) the number of bacteria left alive after a certain time, and (2) the number killed (which of course is easily ascertained if we know the number we started with). Madson and Nyman, Chick, Earle Phelps, and others in their valuable work on the measurement of the rate of death of bacteria have not yet come to a final conclusion. Miss Chick suggested that disinfection was similar to a unimolecular reaction and that it takes place in one direction only, namely, live organism + active disinfectant \rightarrow dead organism + active and inactive disinfectant. The velocity of the reaction would then be represented by the equation $\frac{db}{dt} = kb$, where b is the number of organisms present at any time in unit volume, and k the velocity constant.

Values of k which should be constant, depending only on the kind and concentration of the disinfectant and on the temperature and bacterial species, do not give concordant results. One must therefore attack the problem once more and find whether this equation meets the case and whether this is really the actual mechanism of the reaction. We have in this equation three factors to consider: the organism, the disinfectant, and the time. The time is known and controllable; we may therefore regard this factor as determined. The second factor is the organism. Microorganisms as a whole are susceptible to change. This is recognized in the Rideal-Walker test, which cultivates a known organism for a known time on a definite medium at a given temperature. This culture produces what might be called a community of organisms whose age, capabilities for work, etc., are just as variable and heterogeneous

as in a community of human organisms, but gives on the whole a fair average. Earle B. Phelps wishes to make a reduction of 50 to 75 per cent on the total number present as a standard. This is obviously wrong, as the "sick" or "starved" bacteria will die first and leave the hardy and virile ones which might need stronger and more prolonged doses of the disinfectant to kill them. This equality of the organism is the chief corner-stone of the unimolecular law hypothesis. If the bacteria all budded or spored at the same time, if the medium round them was perfectly homogeneous, if their parents were equally strong, if there was no heredity, if they never kept food or light from their neighbors, if the disinfectant were dealt out in equal measure to each microbe, if the osmotic pressure of each personage was the same, if each body was of equal area, if there were no deformed, if in fact microbes were ions or atoms, then the equation $\frac{db}{dt} = kb$ might perhaps hold true. In fact, as disinfection varies with the concentration the unimolecular formula is not applicable, and, as shown by Earle Phelps, k in the above formula is not a constant and has to be replaced by the more complicated expression KC^n where n is an exponent representing the order of the reaction.

To show what is meant by the changeability of a set of organisms, one might mention the change in the toxic value of diphtheria when grown in guinea-pigs or on agar, or the change in cholera vibrios if kept in water, or the resistance of anthrax after 10, 20, and 30 hours' culture. If an entire community can change, how much more can variations be found inside the community and especially in those extremely variable factors, namely, those of life and death.

While still dealing with the same point, it may be noticed that dead bacteria are not necessarily removed from the sphere of action. It is not to be supposed that the action of killing and making alive is a reversible one, but it has not been proved that dead bacteria are not still affected by the germicide. Rather, on the contrary, it has been shown that at least in a few cases sterile organic matter present lowers the Rideal-Walker figures, and certainly dead bacteria come within the scope of the term sterile organic matter.

The unimolecular equation assumes that no such disturbing factor should be present and this can be true only when the disinfectant is in such large excess that the addition of even very small traces of organic matter can make no difference, and this is not experimentally possible.

We have now to deal with the third and last factor, the disinfectant. It has been assumed, up to the present, that all disinfectants act in the same way. There is no foundation for this belief.

The first essential of a disinfectant is that it must come in contact with the organism. We must assume that this takes place and discuss the next proceeding from this premise. Death as a result of exposure to sunlight or to desiccation is an example of this essential factor, while the futility of suspending an insoluble so-called disinfectant block in or above a urinal is also obvious.

Although the organism has to be killed by this contact, we are not at liberty to assume that death takes place in one manner only. HgCl_2 does not necessarily kill the same way as $\text{C}_6\text{H}_5\text{OH}$, or again as KMnO_4 . We can make a rough hypothetical classification of disinfectants.

a) Oxidizers.—Here death is caused by direct oxidation and the result in the medium is a loss of organic matter with an increase of CO_2 . The Rideal-Walker figure will not be altered by the addition of silica, except in the case of very unstable oxidizers, i.e., peroxides or hypochlorous acid, where the presence of a finely divided substance catalytically hastens the liberation of free oxygen. Most disinfectants of this class exhibit complications due to (*b*).

b) Ionic poisoning.—H. Crooks (at R.S. Soirée, June 14, 1911) has shown that metals have a germicidal action within a measurable range of their apparent free surface which can be attributable only to the ions of the metals going into solution due to their electrolytic solution pressures. The germicidal value is not necessarily proportional to the electrolytic solution pressures, since the germicidal value of each ion is not necessarily the same. Hence adding silica, etc., and inorganic matter will not decrease the value; adding certain organic matter, i.e., matter that will be affected by the ion, will lower the value. Adding organic matter that is not affected

by the ion, but which acts only as a diluent and increases the ionization will probably alter the germicidal value favorably. HgCl_2 , $\text{Hg}(\text{CN})_2$, AgF , etc., are probable examples of this class. To this class we may add all substances that pass into the system of the body of the organism whether as ions or not, since Kahlenburg has shown that all reactions are not necessarily ionic.

c) *The emulsoïd class*.—This is mainly exhibited in the tar derivative disinfectants. Here adsorption on the surface of the body takes place and causes death by altering that surface either by subsequent combination with the tissue or formation of a solid solution, or by purely surface tension effects with formation of a skin which alter the osmotic pressure of the membrane. Methylene blue is observed to stain the skin in this manner and after a time the organism dies. Methylene blue is therefore a disinfectant and can be taken as a type of the surface action class. Here the addition of foreign matter will in general alter the Rideal-Walker figure whether the matter is organic or inorganic.

d) *Ultraviolet light*.—Action unknown.

e) *Heat*.—Complicated by increased oxidation and other chemical changes.

It must be understood that possibly no disinfectant is limited in its action to any one of these arbitrary divisions. Even if the organism were a constant factor it would indeed be strange if such a variety of actions followed a unimolecular law. It is extremely doubtful if any of them agree with that hypothetical reasoning in practice, while they certainly do not in theory.

Another point that has attracted a great deal of attention of late years is the influence of foreign matter on the germicidal value. Foreign matter may be either inorganic or organic, soluble, colloidal, gelatinous, or quite insoluble and massive (opposed to colloidal), of known composition or of unknown structure and variable composition. The addition of such matter may influence both the velocity of disinfection and also the actual amount of useful work a definite quantity of disinfectant can do. It is our duty from a practical standpoint to investigate whether any useful end will be attained by adding any foreign matter when the Rideal-Walker test is performed.

Apart from the practical utility of the addition, there is this technical disadvantage to the process. Foreign matter, if it affects the Rideal-Walker coefficient, generally lowers it. Sommerville and Walker have shown the lowering of value of a number of disinfectants when tested in the presence of such substances as urine, blood, gelatin, starch, etc., and in the method suggested by Chick and Martin a general lowering in value is due to this cause. Let us consider two disinfectants A and B and suppose them to have Rideal-Walker coefficients of 23 and 15 respectively. The Chick and Martin feces method might come out at 5.9 and 5.8; it is clear that a variation of one unit in determination will affect the second series a great deal more than in the first lot. It is known that no two determinations come out quite the same, and the reason for this has been shown above when dealing with the lite factor. We would consequently be wiser to accept the larger figures than the smaller ones, as a mere precaution of safety.

The addition of foreign matter, either organic or inorganic, of unknown and variable composition, such as feces, blood, milk, urine, etc., is therefore not to be advised. Even in the Lancet method slight variations in the figure may easily be obtained unwittingly in a well appointed laboratory from which reliable figures usually come, since as the composition alters so does the figure, and although according to this technic the alteration is not great, differentiation between any two disinfectants is no longer possible with guaranteed certainty.

We have discussed the possible methods of disinfection and apparently we can reduce them to the following independent reactions which may take place at the same time, one after the other, or one with another. Observing the types in slightly more detail we see that:

Oxidation of organic matter depends upon the structure of the substance attacked and on the intensity of the oxidation. Hence adding any definite oxidizable matter only gives a figure for that single substance and not for any other; e.g., the oxidation of gunpowder is not comparable numerically to that of mercury but both are oxidation changes and are consequently placed in the same category in physics and chemistry. If the matter is not oxidizable

then there may be catalytic surface decomposition. This depends on the area of the surface of the substance added and probably on its state of division resulting in the liberation of oxygen without necessarily doing effective work. The stability of the disinfectant is, however, assumed if one is bought having a guaranteed Rideal-Walker figure, since if the Rideal-Walker is taken just before use it must have its original value, otherwise the guaranty is useless.

Ionic poisoning.—Here death ensues presumably just as it does in the human organism, by some type of chemical action between the disinfectant and some part of the organism's metabolism, which results in the death of the organism, possibly without such further change as occurs in the oxidation class. It is possible, however, that further action does take place even after death. This would almost certainly occur if the cell wall, for instance, were attacked, for the organism would probably die when only a small part had been destroyed or altered, while the attack would still go on after death. If, however, an important enzyme only were attacked the organism would die when the enzyme was all destroyed and no further change would result. Adding inorganic matter such as silica alumina (colloidal) would not affect the figure to a large degree, although ionic precipitation of the colloid would probably take some of the disinfectant with it. The organic matter that one may add may be affected by the ion or it may not; if the organic matter is not colloidal the reaction will probably take place fairly slowly, as is usual with most organic reactions, and consequently very unreliable and fluctuating results will be arrived at.

Emulsoid class.—Under this class we include all those disinfectants that act by external application only. As we have seen, death may ensue either by alteration of the osmotic pressure, possibly the case with alcohol, by combination with the membrane, by the formation of a solid solution with the membrane, or by being adsorbed by purely surface tension effects. Now it is in this class of disinfectants that most of the velocity work has been done, and it is especially to suit this division that the proposed modifications of the Rideal-Walker method have been proposed. From Chick's work it appears that adsorption certainly does occur in

certain cases. Now inorganic substances exhibit this property also, e.g., charcoal. Travers has shown that the physical nature of the charcoal has a great effect on the adsorption coefficient. Adding charcoal, etc., is consequently similar to adding more bacteria, with the advantage that the charcoal can be made slightly more homogeneous in structure than the bacteria and this lends itself better to the measurement of velocity. We must not consequently give up performing the Rideal-Walker test with bacteria and use charcoal instead for this restricted class of disinfectant, first, because it is bacteria we wish to deal with in practice, and second, because we are not certain that the process is a pure adsorptive one and that toxic influence does not play a part in the proceedings according to our second class. It has been shown that if

x = quantity of disinfectant adsorbed

a = area of adsorbing surface

c = concentration of disinfectant

$\frac{x}{a} = Kc^n$ where k and n are constants for the system. a is usually measured by the weight of the substance taken.

If, however, we are dealing with cocci, $W \propto r^3$, assuming the cocci homogeneous (which of course they are not), while $a \propto r^2 \therefore a \propto W^{\frac{2}{3}}$. Now this is the equilibrium saturation even if death is due to adsorption only. It has not been shown, however, that microorganisms do not die before saturation is complete, and consequently it may be that a Rideal-Walker with organisms will have a higher value than a modified Rideal-Walker with charcoal in which the end point is measured by the presence of free disinfectant in the solution.

From the above we have seen that we cannot advantageously add matter that has a peculiar chemical structure. We must consequently add matter that remains indifferent to all disinfectants from a chemical standpoint. In its physical aspect, however, the mechanism of a few disinfectants resembles at least in the final stages of the process that one known as adsorption. This we can measure with alumina or, if strong oxidizers are excluded, with standard silk.

As we have seen above, adsorption may not be the only factor concerned, so that a high value for the ratio $\frac{x}{a}$ does not necessarily indicate a high Rideal-Walker, and the reverse is certainly not the case.

Anderson and McClintic¹ suggest modifications of the Rideal-Walker technic without materially altering the results. They prefer *B. typhosus* as the test organism and also admit the desirability of not adding any organic matter in suspension during the duration of the test. The modifications are of only a minor character and are (1) raising the standard temperature to 20° C; (2) fixing the proportion of culture to disinfectant to 0.1 c.c. in 5 c.c. of the disinfectant dilution; (3) taking the mean figure between the strength and time coefficients in a 15 minutes test instead of only one ratio falling within such range.

These changes might easily be accepted by all workers were it not for the fact that the original method is now so widely used in England, India, and her colonies, and we would suggest that at the International Congress in Hygiene, meeting in the United States in 1912, the matter might be finally settled. The temperature 20° C. is somewhat high for general disinfection in northern latitudes and it is as easy to work at the original temperature limits of 16°-18° C. in summer weather as to raise the temperature of the room or reacting bath during winter testing. The general effect of the higher temperature is to give figures which show that disinfection has proceeded more rapidly and that disinfectants have greater germicidal properties at the higher temperatures, so that a greater margin of safety must be allowed when practical disinfection in cool weather is required.

In altering the proportion of culture to disinfectant, care should be taken that the gauge of the wire in the loops used should be sufficiently thick to be rigid and at the same time admit of rapid cooling after sterilization, unless a series of loops as suggested are employed. At the same time accurate results are now certain when one loop only is used in a test, as no two loops can deliver mathematically exact doses.

¹ *Jour. Infect. Dis.*, 1911, 8, p. 1.

As to the adoption of a mean figure instead of the lowest ratio in a harmonious curve, this figure will as a rule be higher than the figure at $2\frac{1}{2}$ minutes and smaller than that at $12\frac{1}{2}$ or 15 minutes. As the aim of disinfection is to insure death in the minimum time, one would be inclined to select the disinfectant which gives the highest figure at the $2\frac{1}{2}$ minutes' interval. The figure obtained at $7\frac{1}{2}$ minutes is very close, if not identical, with that obtained from the mean between the maximum and minimum times included in the testing period.

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THE CHANGES IN INFLUENZAL PNEUMONIA.*

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During epidemics of influenza, pneumonia occurs in about 5 per cent of the cases and the mortality ranges from 15 to 50 per cent. Before Pfeiffer's discovery of the influenza bacillus it had been noted that pneumonia was common during the epidemic periods, and efforts were made to determine its cause. Chiefly streptococci and pneumococci were found in the lungs and though by some they were considered the cause, by most writers they were thought to be secondary invaders. After Pfeiffer's work¹ the problem resolved itself into a correlation of bacteriological results and anatomic alterations. Pfeiffer declared that the influenza bacillus could be a cause of pneumonia, since it was found in some cases practically pure in the lung parenchyma. Anatomically many of these pneumonias were entirely catarrhal and seemed to correspond to the ordinary lobular pneumonia. Leichtenstern² makes the following statement concerning this type of pneumonia: "The description of influenza pneumonia is but a repetition of what we have long known regarding the anatomic course of a pure catarrhal

* Received for publication February 17, 1912.

¹ *Ztschr. f. Hyg. u. Infektionskr.*, 1898, 13, p. 357.

² Nothnagel, *Encyclopedia of Practical Medicine*, 1905, p. 611.

pneumonia. As the inflammation proceeds from the bronchi into the pulmonary tissues there arise, as always under such conditions, lobular areas of inflammation which either remain separated by tissues containing air or coalesce to secondary lobar areas whose origin from lobular foci can still be recognized. The cut surface is quite smooth and on pressure drops of yellow pus ooze from the severed bronchi. Microscopically the whole appearance is one of catarrhal suppuration in optima forma. The alveolar lumina and septa, as well as the peribronchial connective tissue, are so infiltrated with round cells that apparently the lung structure is entirely obliterated. In the alveoli surrounding these purulent infiltrations, besides containing round cells, the alveolar epithelium is much swollen. In preparations stained by Weigert's method the absence of fibrin (or its occurrence at most in traces) in the areas of infiltration is very evident."

This lobular type of pneumonia, however, was by no means the only form that complicated influenza. According to many writers the croupous form often occurred and was noted by some to be more common than the lobular (Birch-Hirschfeld, Naunyn, Rollinger, Marchand, Weichselbaum, Menetrier). The question became complicated apparently for two reasons: first, epidemics of genuine lobar pneumonia existed simultaneously with the epidemics of influenza, as was noted, for instance, by Weichselbaum in Vienna; second, because atypical and mixed forms of pneumonia commonly occurred. These latter varied markedly both clinically and anatomically and naturally were described very differently by different observers.¹ Thus there arose much confusion and difference of opinion, and many points concerning the true character of influenza pneumonia remained unsettled. Quoting from Leichtenstern again: "The reason for this difference of opinion is obvious. It depends upon the fact that at the bedside it is often impossible to differentiate between the two forms (lobular and lobar), and that the anatomic differential diagnosis is by no means always easy and certain. It is in influenza especially that transition forms of lobular and lobar infiltration occur whose anatomic character, whether catarrhal or croupous, is often extremely difficult either macro-

¹ Kuskow, "Pathologische Anatomie der Influenza," *Arch. f. Path. Anat.*, etc., 1895, 139, p. 406.

scopically or microscopically to recognize." The cause of these atypical catarrhal-croupous or mixed pneumonias, while not entirely clear, would seem to be the existence of mixed and secondary infections, for in such cases pneumococci or streptococci or both, with or without influenza bacilli, are not infrequently found. It should be stated that much of our data on influenza were obtained during the epidemic of 1889-90 and were almost purely anatomic in character, the bacteriologic studies at this period being incomplete. Since that epidemic the influenza question has been further confused on account of the fact that the terms "influenza" and "grippe" have been applied without bacteriological examinations to almost every clinical condition that bears any resemblance to true influenza, including the common epidemics of colds. Many of these infections we now know are caused not by the influenza bacillus, but by streptococci, pneumococci, *M. catarrhalis*, etc. The confusion regarding the etiology of influenza or grippe naturally was extended to the complicating pneumonias, and consequently the term influenzal pneumonia was applied to lesions with which the influenza bacillus had nothing to do.

The above statements are sufficient, I think, to indicate the difficulty of obtaining from the literature a clear conception of influenzal pneumonia and to give an idea of the confusion that exists concerning this disease. I have therefore been led to present some data on this subject which have been acquired from the study of a number of cases of influenzal meningitis¹ associated with pneumonia. Five cases came to autopsy. In four, in addition to the pure influenzal meningitis, were definite pneumonic lesions in which the influenza bacillus occurred nearly pure or as the predominating organism. The respiratory tract also, as would appear from the clinical histories, was the probable primary seat and the atrium of infection. In three instances the influenza bacillus was found in the heart's blood practically pure. There can be no doubt, therefore, that the cases were severe infections with the influenza bacillus and that the alterations in the lungs may be regarded as typical of influenzal pneumonia. A somewhat detailed description of these lesions is therefore given and the results are

¹ These cases of meningitis were reported in *Am. Jour. Dis. Children*, 1911, 1, p. 249.

compared with the observations of others on pneumonia associated with influenza meningitis and also on bronchopneumonia of other origin, especially that complicating the respiratory type of influenza.

The five cases examined were all children one year of age or less. Clinically a definite history of "colds," preceding the meningeal trouble, was obtained in four cases, and bronchitis or bronchopneumonia was recognized usually a short time before the meningeal symptoms and several days before death. The pneumonia was manifested by fever, harsh breath sounds, areas of dulness, fine and coarse rales, etc. In general, both clinically and pathologically, the cases are very similar and individual statements or descriptions, except in certain instances, need not be given.

From a careful review of the alterations in the lungs the following account is written. Macroscopically in four of the five children there are definite pneumonic regions. Both lungs are affected about equally, and the consolidated portions occur chiefly in the lower lobes and in the posterior parts. In two cases the upper lobes are likewise involved. In no instance are there pleural adhesions either between the lobes or in the pleural cavities. Over the consolidated portion of the lungs as a rule the pleura is granular and covered by a thin delicate layer of fibrin. There is no fluid in the pleural cavities in any case. In one instance small hemorrhages are seen just beneath the pleura.

On section the lungs are moist, but not strikingly bloody, even in the lower parts. The distribution of the regions of consolidation is similar in most cases and the irregular lobulations are easily seen. The pleural surface is distinctly elevated over the involved parenchyma and here and there, especially around the consolidated lung tissue, are often bluish, depressed regions of atelectasis, variable in size. Single consolidated lobules may be found, but in two cases they have coalesced, forming consolidated masses one to several centimeters across in the posterior and lower parts of the lung near the hilus. These consolidated parts sink in water and are absolutely airless. The surfaces made by cutting them are smooth and do not present the granular appearance of lobar pneumonia. The color is reddish or yellowish gray. This pneumonic lung tissue is often sharply marked off from the surrounding regions, especially

by the fibrous septa, and for the most part lies located immediately under the pleura.

Adjacent and usually anterior to these confluent pneumonic regions are others less completely solidified and having a mottled red and gray color. This mottling, as seen on the cut surface, is due to small solid gray areas one or two millimeters across, separated by slightly darker zones. Such regions are conspicuous on section of the lungs and are far more extensive than the completely confluent regions. From their centers, on slight pressure, there exude small droplets of pus. About these mottled regions, especially anterior and extending well forward sometimes to the anterior margin of the lung, the tissue contains much more air, does not sink in water, and is darker red in color; here are only a few gray points representing small bronchi surrounded by a narrow zone of pneumonic lung. In places along the anterior margin the lung tissue is sometimes slightly emphysematous, but this is not constant. Fibrin plugs are not noted in the bronchi. In the larger bronchi abundant mucinous purulent fluid is always found and the mucosa is diffusely red. This condition extends into the trachea and involves the upper respiratory tract generally.

For microscopic examination pieces were obtained from different parts of the lungs, sectioned in paraffin and stained with hematoxylin and eosin, Van Gieson stain, Weigert's elastic fiber stain, polychrome methylene blue, methyl green-pyronin mixture, dilute carbofuchsin, and Giemsa stain.

In the consolidated lung tissue as a rule the alveoli and bronchial tubes are filled with a rich exudate. At the margins of the consolidated portions it is evident that the process is essentially lobular because the infiltrated regions surround the bronchi and are separated by tissue which contained air during life. In places a branching distribution of the pneumonic areas suggests the successive invasion of the various parts of the lung along the bronchial tubes. In some sections there are large bronchi with intense mural infiltration surrounded by a narrow marginal zone of pneumonic acini. The epithelial cells lining the bronchial tubes are often desquamated, lying free in the lumen singly or in small clusters. They may show evidence of marked degeneration. In the epithelial

layer may be seen polymorphonuclear leukocytes and an occasional plasma cell, apparently fixed while in the act of wandering outward into the lumen. Accumulation of leukocytes just under the epithelium is not noted in the sections. The small blood vessels and capillaries in the wall are usually intensely hyperemic.



FIG. 1.—Photomicrograph showing the extensive plasma cell infiltration about the bronchial tubes.

Strikingly conspicuous, especially as shown by the methyl green-pyronin stain, is the extensive plasma cell infiltration in the walls of the bronchi (Fig. 1). This is seen both in the large and small bronchi and appears to be chiefly in the peribronchial lymph spaces. About some of the bronchi the exudate consists of plasma cells almost entirely, the small mononuclear and polynuclear cells being but few. Plasma cells occur also in the connective tissue spaces about small blood vessels, and, as stated, may be seen in the

epithelium. They may be found free in the bronchial lumen and in abundance about the larger blood vessels in the region of the bronchi and along the fibrous septa in the lymph channels. Evidently they do not wander into the alveoli for they are not found here. In one case in which there was clinical evidence of bronchopneumonia for a few days only and in which consolidation was not extensive, the plasma cells about the bronchi are present in moderate numbers only.

The alveolar walls are congested and here and there is desquamation of the lining cells of the alveoli. In one case there are in the alveoli a few giant cells evidently formed from the desquamating epithelium.

The exudate in the bronchi and alveoli contains, as a rule, many polynuclear leukocytes; in places, indeed, there seems to be little else. In the larger bronchi, in addition to the numerous pus cells, a few plasma cells, as already stated, may be seen at times. Desquamated epithelial cells are not uncommon, also other mononuclear cells. The latter are probably what Mallory has called mononuclear leukocytes and which were found so commonly by Councilman, Mallory, and Pearce in bronchopneumonia in diphtheria. In the early stages, as was noted especially in one case, there is in the larger bronchi an abundance of tenacious mucoid secretion which, in smears, reveals mucinous material with numerous leukocytes and influenza bacilli. In the alveoli, as stated, polynuclear leukocytes are the chief cellular elements. Plasma cells are practically never found here, but mononuclear cells may be moderately abundant at times. Large, swollen, desquamated epithelial cells containing brown or nearly black pigment are found almost constantly. In some parts of the lung, especially those bordering on the involved regions, these large cells may be very numerous and frequently contain many ingested polynuclear and mononuclear cells. They often show evidence of fatty degeneration.

The occurrence of fibrin in influenzal pneumonia has been the subject of numerous remarks in the literature. Some authors state

¹ That plasma cells may migrate through tissues, epithelium, etc., has been shown by Schridde, *Fol. haematologica*, 1907, Supplement, 3, p. 286.

that the fibrin is absent or occurs in small amounts only. Others report finding it in large amounts especially in the mixed or cellular-fibrinous type. In the cases here examined fibrin is found in all, but on the whole it is not abundant. Its distribution in the tissues is limited almost entirely to the alveoli immediately around the larger bronchi, where it occurs in rather large masses in the centers of the distended alveoli. Occasionally an isolated mass is seen in the lung tissue at some distance from a bronchus, especially in the newly involved portions. In the bronchi it is often seen in considerable quantities covering the epithelium and appears here, as a rule, as a fine meshwork and not as solid hyaline masses.

Red blood corpuscles are commonly found in the bronchi and in the alveoli, especially near bronchi where fibrin is abundant. Nowhere are definite hemorrhages seen. In the vessels not infrequently are thrombi of considerable size, partly or entirely occluding the lumen. The vessel walls are not involved and no changes except swelling of the endothelial cells lining the arteries are seen. Often in the lumen of the smaller vessels are masses of cells, many mononuclear but chiefly polynuclear in type, which are sometimes imbedded in fibrin.

The interstitial tissue shows some cellular infiltration in places, though not to an extensive degree. As stated, plasma cells are found in abundance along the connective tissue septa. Here and there, especially under the pleura, may be seen accumulations of round and polynuclear cells in the lymph channels. No increase in connective tissue appears in the septa, pleura, or in the alveolar walls. Beginning organization was noted in one case in the exudate in a small bronchus.

BACTERIOLOGY.

The influenza bacillus was found in pure culture in the heart's blood in three cases. Smears of the bronchial and lung exudate, sections of the lung tissue suitably stained, and cultures on blood media were examined with reference to bacteria. Generally the influenza bacillus was found as the predominating organism. In one case invasion of the lung by saprophytes after death had occurred to such an extent that pure influenzal cultures were not obtained, but in the smears many typical influenza bacilli were

seen. A few pneumococci or streptococci and at times both were found in the bronchi and lung exudates in culture, and in the stained sections cocci were often seen mixed with the bacilli. This is not surprising in view of the fact that these organisms are constantly found in the mouth, throat, and sputum and may easily be inhaled into the lung tissue. Their insignificant numbers and the general preponderance of influenza bacilli make it appear very improbable that they played any important part in the process. They were undoubtedly secondary invaders and have as much or as little significance as the not uncommon finding of a few streptococci or influenza bacilli in the lung exudate in lobar pneumonia.

In the sections stained with Giemsa stain immense numbers of bacilli were often seen in the leukocytes, especially in the bronchi. They were much less commonly found in the alveoli. Coccus forms also were seen in such preparations, confirming the results obtained by cultures. Occasionally there occurred in the bronchi masses of small bacilli staining blue in the hematoxylin preparations. I was never able to see influenza bacilli in the epithelial cells as has been reported by some observers.

In view of these results, it would seem that in the larger bronchi the process is generally limited to an inflammation of the mucosa and peribronchial tissue with little involvement of surrounding alveoli. It is in the smaller bronchi, however, that the process appears most intense. Here is seen in every case extensive peribronchial cellular infiltration, chiefly of plasma cells, a fact which points to these parts of the bronchial tree as being the chief seat of infection. Undoubtedly the influenzal infection in these cases which began as "colds" passed down the respiratory tract, localizing in these smaller bronchi and existing for a time as a bronchitis and peribronchitis. It later extended farther along the tubes and also laterally into the adjacent alveoli (chiefly the former), producing the well marked lobular pneumonia.

A review of the literature shows that pneumonia often has been found associated with influenzal meningitis. The lesions have not, however, been very carefully described, and usually the mere statement of an associated bronchopneumonia appears in the reports. At the present time 60 cases of influenzal meningitis have

been reported, and autopsies have been made in about one-half of this number. I have reviewed the records of 26 cases in which statements appear concerning the condition of the lungs, and in 20 there was found lobular pneumonia. Lobar pneumonia was not found in any. So far as the descriptions indicate, there seems to be a striking similarity in all. Ghon¹ describes concisely the lesions in one of his cases as follows: "In both lower lobes are scattered, elevated, pea-sized, nodular, grey-red, air-free regions. In the bronchi is a mucopurulent secretion and the mucosa is red and swollen." In his second case the lobular pneumonia was confluent in places. Microscopically the alterations were those of ordinary bronchopneumonia. Leukocytes were abundant and some fibrin was present. In the centers of the nodules was often some dissolution of alveolar septa. The reports on the bacteria found in the lungs are also usually incomplete. Cultures were made in only a few cases and the statements are mostly based on observations of microscopic sections. Generally small bacilli (influenza), intracellular and extracellular, have been found mixed with cocci, usually streptococci. In general the above statements apply to all the cases in the literature so far as the records permit one to say. They are also in complete accord with the cases described in this paper.

The most complete accounts of the pathology of pneumonias occurring in the ordinary acute respiratory form of influenza have been given by Pfeiffer,² Beck,³ and Leichtenstern.⁴ The type that seems to occur frequently and in which Pfeiffer especially found influenza bacilli, pure or nearly pure, in the lung parenchyma, is that of a catarrhal bronchopneumonia. As stated, mixed forms and lobar pneumonia are at times common during epidemics of influenza, but the authors cited claim they are usually if not always due to mixed infections. According to their descriptions of influenzal bronchopneumonia the lung surface has a mottled, light-gray and flesh-red color and shows little or no fibrin. The lobular pneumonic regions may be found in all lobes but occur more fre-

¹ *Wien. klin. Wchnschr.*, 1902, 15, p. 667.

² *Ztschr. f. Hyg. u. Infektionskr.*, 1893, 13, p. 357.

³ Kolle and Wassermann, *Handbuch der Path. Microorg.*, 3, p. 382.

⁴ Nothnagel, *Spec. Path. u. Therapie.*, 4, p. 83.

quently in the lower, and the bronchi are filled with thick pus in which the influenza bacilli, often mixed with cocci, are numerous. Round cell infiltration is common about the bronchi and in the alveolar walls. Beck states that the catarrhal process in influenza advances down the passages in a branching manner, thus producing the lobular distribution. Comparing the description of this type of pneumonia, especially as given by Pfeiffer, with the pneumonia encountered in the meningitis cases, one must admit that no characteristic features exist which will differentiate them.

Lord¹ has also described a number of cases of influenza pneumonia which were confirmed by bacteriological studies and he likewise concludes that the influenza bacillus does not produce a definite or specific type of pneumonia.

I have assumed, heretofore, that the bacilli found in influenza meningitis and that found in true epidemic influenza are identical. Cohen² contends that there is evidence from conglutination and other tests to believe that they are not alike, and his results seem to have been confirmed recently by Odaira,³ who made extensive agglutination and hemolytic experiments for differential purposes. However, morphologically and culturally they cannot be differentiated and it would appear from the study here made that they produce lung lesions which are indistinguishable from one another. If different at all, they are without doubt very closely related and I am inclined to believe that the difference noted by Cohen may be simply one of virulence.

Not only do the lobular lung lesions found in influenzal meningitis and in true influenza appear to be identical but they agree closely with the acute lobular pneumonia occurring commonly in children, especially during the course of various infectious diseases. The localization generally posteriorly and in the lower lobes, the lobular distribution, the nodular, at times confluent, elevated, consolidated regions, variable in size with adjacent small atelectatic areas, the cellular character of the exudate with its scant fibrin content, and the distribution of the bacteria all agree even in

¹ Osler, *Modern Medicine*, 2, p. 272.

² *Ann. de l'Inst. Pasteur*, 1909, 23, p. 273.

³ *Centralbl. f. Bakt.*, 1, Orig., 1911, 61, p. 289.

minutest detail with our conception of acute catarrhal or lobular pneumonia as given in our standard textbooks.

In none of the cases of meningitis was the pneumonia associated with such complications as abscess formation, gangrene, extensive necrosis, hemorrhages, bronchiectasis, etc., as noted in influenza pneumonia by some authors.¹ This may be due to the fact that sufficient time did not elapse for such processes to go on, the patients dying relatively early from the meningitis. Bronchiectasis has been noted in chronic bronchopneumonias, especially following influenza, measles, and pertussis, in which conditions the influenza bacillus has often been found. Boggs² reports a series of such cases and Vogt³ calls especial attention to this point. At times there was noted a breaking down of the walls of bronchioles and alveoli with accumulation of pus cells, disintegration and disappearance of the epithelium. Had the meningitis patients continued to live, it is possible that such processes might cause bronchiectatic cavities to form, but one would not expect to meet them in an acute condition.

A word might be said concerning the hypostatic paravertebral pneumonia as described by Gregor and later and more completely by Bartenstein and Tada.⁴ This form of pneumonia, according to these authors, develops chiefly in the posterior and lower parts of the lung and results from circulatory disturbances which may have a variety of causes (form of breathing, malnutrition, etc.). Infection plays no etiological rôle whatever. Though bacteria are often found in the tissues, they are purely secondary invaders, as evidenced by the fact that in the very beginning of the process they are not found in small pneumonic regions nor in the advancing periphery of the larger ones. Another feature of paravertebral pneumonia is a general bloody and hyperemic condition of the lung tissues as contrasted with the more local hyperemia of the infective form. As regards the cases of influenzal pneumonia above described, marked hyperemia was not a striking feature, the tissue as a rule being grayish red. In sections the bacteria were found everywhere, being present in the margins of the consolidated por-

¹ Paltauf, *Wien. klin. Wchnschr.*, 1899, 12, p. 576.

² *Bull. Johns Hopkins Hosp.*, 1905, 16, p. 288.

³ *Fortschr. d. Deutsch. Klin.*, 1911, p. 473.

⁴ *Beiträge zur Lungenpathologie der Säuglinge*, Leipzig, 1907.

tions as well as in the bronchial exudate. Again, the bronchi are intensely and probably primarily involved in these cases, as shown by the marked peribronchial infiltration, whereas in the paravertebral form the parenchyma is first involved and only later are the bronchi affected. For these reasons I think it is evident that influenzal pneumonia does not belong to the hypostatic paravertebral type.

SUMMARY.

Influenzal bronchopneumonia occurs in a large proportion (78 per cent) of all cases dying of influenzal meningitis. As a rule it develops early and may precede the appearance of the meningeal symptoms.

The influenza bacilli are found in the lung and bronchial exudate but usually mixed with a smaller number of other organisms (streptococci, pneumococci, etc.). The lungs are probably the atrium of infection in many cases though not in all.

The pneumonia is always lobular in character and does not appear to differ morphologically in any essential respect from the lobular pneumonia commonly associated with the respiratory type of influenza or from that associated with other acute infectious diseases.

A BIOMETRICAL STUDY OF MILK STREPTOCOCCI.*†

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A comparative study of the fermentative reactions of milk streptococci was begun in February, 1911, at the suggestion of Professor C.-E. A. Winslow, and, with occasional helpful criticism from him, completed early in June, 1911. Through the courtesy of Dr. William H. Park of the New York Research Laboratory, 100 strains of streptococci were obtained from milk plates made in the routine milk examination conducted by Dr. Schroeder. Most of these plates were made from samples obtained at the farms and dairies, and shipped on ice to the Research Laboratory. But one strain was used from each plate, making, presumably, 100 sources for the 100 strains isolated. Fishings from the agar milk plates supplied by Dr. Schroeder were grown in milk, transferred to agar streaks and then again to milk, next to slant agar for two or three days (until a good growth was secured), and then transferred to the special media. These included eight of Gordon's¹ nine test substances: neutral red for reduction; milk for coagulation; and for acid formation, lactose, saccharose, salicin, raffinose, mannit, and inulin. Sugar-free broth was used in making 1 per cent media of the last six substances. Microscopic examination (methylene blue) was made at every stage. Only those strains were used which produced on agar the characteristic veil-like growth, and which showed in liquid media chains of four or more cocci. Chains of 40 were the longest observed. With a few exceptions the early stages of isolation, owing to lack of incubator space, were conducted at room temperature; slow-growing organisms were reincubated at 37° C. This latter temperature was used for all the agar slants and for all the special media. After three days at 37° C. these special media (with controls) were titrated to determine the amount of acid formed. Five c.c. of liquid and 45 c.c. of distilled water were

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¹ Supplement, *Thirty-third Annual Report, Local Government Board*, 1903-4.

titrated in the cold against $\frac{n}{20}$ NaOH, using phenolphthalein (three drops) as an indicator. Two controls were titrated with every lot, and their average results subtracted from those recorded for the inoculated tubes. As a color standard the pink in the top line of the color chart frontispiece of Winslow's *Systematic Relationships of the Coccaceae* was used. Each agar tube used in inoculating the special media was reincubated (for three days) and then examined microscopically. Purity in this agar tube was taken to indicate purity in the eight subcultures previously made from it.

The 31 strains first isolated were not transferred directly to the special media, owing to an unavoidable delay in the preparation. They were subcultured on agar for one week, but good growths were obtained before transferring to the test substances mentioned. In this lot, however, are found all of the 11 strains fermenting salicin only; these will be mentioned later.

RESULTS.

The results for the 100 strains are given below (Table 1). The first column gives the series numbers, arranged in order of isolation. The records of acidity represent, as stated above, the difference between the value obtained for each culture and two controls incubated at the same time under the same conditions. Coagulation of the milk and reduction of neutral red are indicated by the plus sign; the lack of either by a minus sign.

In the second table the results, except those for milk and neutral red, are grouped together in percentage acidity classes. In each test substance there were two types of organisms: one yielding little or no acid, or even causing an alkaline reaction; and a type yielding between 1.5 (approximately) and 8.5 per cent acid. The exact intermodal point for each substance is indicated by an asterisk.

It is of course impossible to consider a different intermodal point for each substance; therefore 1.5 per cent has been selected as a basis for comparison, and all strains falling below 1.5 per cent acid are considered non-fermenters. The acid-forming strains are indicated by a plus sign (Table 3), the non-acid strains by a blank. It will be observed that on this basis a large number of strains

TABLE I.

SERIES NUMBER OF STREPTOCOCCI	PERCENTAGE NORMAL ACID FORMED IN						COAGULA- TION OF MILK	REDUC- TION OF NEUTRAL RED
	Lactose	Saccha- rose	Salicin	Raffinose	Mannit	Inulin		
1	-0.4	4.0	4.0	0.1	1.0	0.1	-	-
2	2.3	3.0	3.7	0.2	2.4	3.4	-	-
3	0.1	0.7	0.2	0.5	-0.1	-0.3	-	-
4	0.2	0.3	2.8	0.4	-0.5	-0.4	-	-
5	2.5	2.3	4.8	0.0	2.5	3.3	-	-
6	0.3	-0.9	4.4	-0.1	-0.4	-0.3	-	-
7	0.6	-0.8	3.0	0.2	-0.4	-0.1	-	-
8	4.0	3.0	0.3	0.1	1.5	-0.1	-	-
9	3.0	0.3	7.0	0.3	-0.7	-0.1	+	+
10	4.5	0.2	0.2	-0.4	-0.3	-0.3	+	-
11	-0.1	0.1	-0.1	0.2	-0.3	-0.5	-	-
12	3.0	2.8	5.0	0.5	3.0	4.5	-	-
13	4.6	3.3	5.8	-0.7	-0.4	0.2	-	-
14	-0.2	0.3	4.3	1.5	-0.4	-0.1	-	-
15	0.3	0.5	3.5	0.2	-0.4	-0.1	-	-
16	0.6	1.7	4.1	0.2	0.6	-0.6	-	-
17	2.7	2.3	2.3	5.1	3.3	0.0	+	-
18	3.0	2.5	1.5	3.7	0.9	1.4	+	-
19	2.0	3.1	4.1	0.5	3.7	4.5	-	-
20	0.1	0.0	3.2	0.2	-0.4	0.0	-	-
21	0.4	-0.8	3.5	0.4	-0.2	-0.1	-	-
22	0.2	0.0	3.0	0.2	-0.2	-0.4	-	-
23	0.1	0.3	3.6	0.5	-0.2	-0.4	-	-
24	-0.2	-0.3	2.6	0.7	-0.3	-0.5	-	+
25	0.1	0.2	3.7	0.3	0.0	-0.3	-	-
26	3.3	2.3	5.7	0.5	2.1	4.1	-	-
27	2.8	2.0	4.0	0.5	3.7	4.5	-	-
28	4.1	3.8	6.0	0.2	1.5	-0.4	-	-
29	0.3	0.0	-0.2	0.2	0.0	-0.1	-	-
30	0.8	0.8	4.5	0.6	-0.1	-0.2	-	-
31	4.8	0.8	6.6	-0.4	-0.2	-0.4	+	-
32	3.3	3.0	0.0	0.2	-0.3	-0.3	+	-
33	2.8	3.1	3.3	0.3	3.0	2.8	-	-
34	3.3	4.1	3.8	0.2	4.2	2.9	-	-
35	3.8	3.6	5.0	0.0	3.5	3.2	-	-
36	2.8	4.0	4.0	-0.6	3.1	2.7	-	-
37	2.5	3.0	1.9	-0.1	2.5	0.0	-	-
38	3.1	0.2	2.4	-0.2	2.6	-0.2	+	-
39	2.4	3.6	3.0	0.4	2.6	2.3	+	-
40	4.2	4.5	2.3	-0.2	0.2	-0.2	+	-
41	2.2	2.8	3.4	0.4	0.3	1.5	+	-
42	2.4	3.4	3.3	-0.1	2.6	0.0	-	-
43	3.2	3.8	3.0	0.2	0.2	2.1	-	-
44	2.6	3.4	3.2	-0.4	3.2	-0.5	-	-
45	4.2	4.8	5.5	-0.2	2.2	-0.5	+	-
46	3.8	4.7	4.1	1.3	0.5	0.2	+	-
47	2.0	0.1	0.7	0.1	0.5	0.4	-	-
48	0.6	0.1	1.1	0.1	0.3	0.2	-	-
49	1.4	0.3	0.2	0.7	0.3	0.2	-	-
50	3.5	1.3	4.3	0.4	0.7	0.6	-	-
51	3.2	2.5	3.0	3.9	3.7	3.2	+	-
52	3.6	3.0	3.7	3.3	3.7	3.8	+	-
53	3.4	3.1	0.5	3.3	-0.3	2.6	+	-
54	3.4	4.1	4.1	3.5	4.1	2.8	+	-
55	4.8	-0.3	2.5	0.3	0.5	0.2	+	-
56	4.4	0.1	3.3	0.3	0.3	0.2	+	-
57	2.4	2.0	0.5	0.5	0.5	0.0	-	-
58	3.4	3.5	1.5	0.9	0.1	2.0	+	-
59	3.4	3.4	5.0	3.0	3.9	4.0	+	+
60	3.2	0.1	0.3	0.0	0.6	0.6	+	-
61	1.0	5.4	4.8	0.6	0.1	0.8	-	-
62	0.8	5.3	4.3	1.1	0.1	0.4	-	-
63	5.0	0.3	7.5	0.2	0.4	0.4	-	+
64	4.6	6.0	3.7	0.1	5.9	0.2	+	+
65	4.4	6.3	5.2	0.4	0.3	0.0	+	-
66	2.6	4.0	-0.3	0.1	0.6	0.2	+	-
67	2.9	3.4	3.1	0.6	0.6	3.0	+	+
68	4.5	5.2	-0.4	0.3	0.5	0.0	+	-
69	5.2	0.2	6.7	0.5	3.2	0.1	+	+

TABLE 1. *Continued*

SERIES NUMBER OF STREPTOCOCCI	PERCENTAGE NORMAL ACID FORMED IN						COAGULA- TION OF MILK	RED- DRESSING NOTED RED
	Lactose	Saccha- rose	Salicin	Raffinose	Mannit	Inulin		
70.	6.3	8.4	0.3	8.3	0.5	0.1	+	+
71.	3.7	4.5	8.8	0.5	4.4	5.9	—	+
72.	2.8	3.2	0.8	0.3	0.0	0.9	—	—
73.	3.2	3.4	4.2	0.1	0.7	4.8	—	—
74.	0.0	3.9	5.2	0.5	0.4	3.5	+	—
75.	2.9	5.3	2.8	0.6	0.9	3.0	+	—
76.	2.3	5.3	4.8	0.6	0.9	3.8	+	—
77.	2.5	3.3	5.5	0.7	1.1	3.9	+	—
78.	2.8	3.8	5.2	0.6	0.7	0.9	+	—
79.	2.0	4.0	4.7	0.5	0.6	3.2	+	—
80.	4.0	5.2	6.5	0.3	0.6	0.2	+	—
81.	4.8	5.1	5.7	0.3	0.5	0.4	+	—
82.	4.1	0.7	0.5	0.9	0.7	0.4	+	—
83.	3.8	2.5	4.5	0.3	0.6	4.1	+	—
84.	0.0	1.7	0.3	0.7	0.5	0.2	+	—
85.	3.0	1.0	4.9	0.0	0.5	4.0	—	—
86.	2.7	5.1	5.0	0.3	2.8	0.6	—	—
87.	0.2	0.7	0.6	0.3	1.1	0.6	—	—
88.	2.5	4.8	4.9	0.4	1.3	3.8	+	—
89.	4.2	0.8	6.2	0.4	0.4	0.7	+	—
90.	2.7	4.8	0.7	3.1	0.2	1.1	—	—
91.	1.8	4.2	0.5	2.8	0.4	2.8	—	—
92.	2.7	4.0	2.2	0.8	0.4	4.9	+	—
93.	1.4	2.8	4.2	0.0	0.6	3.9	—	—
94.	2.0	4.5	0.4	2.6	1.1	5.8	+	—
95.	2.7	4.0	5.0	0.4	1.4	5.1	—	—
96.	2.6	3.3	2.7	3.9	0.5	5.2	+	+
97.	4.7	1.8	6.4	0.4	0.6	0.6	+	+
98.	2.5	4.5	1.2	3.7	0.5	5.3	+	—
99.	4.2	1.4	3.9	1.0	0.7	0.6	+	—
100.	2.0	5.3	3.0	0.6	1.3	1.0	—	—

ferment lactose, saccharose, and salicin. The order of fermentability is salicin, 82 per cent; lactose, 76 per cent; saccharose, 66 per cent; inulin, 38 per cent; raffinose, 13 per cent; and mannit, 27 per cent. Inulin has a high record for a substance heretofore described as not fermented by streptococci.

TABLE 2.
MILK STREPTOCOCCI GROUPED IN PERCENTAGE ACIDITY CLASSES.

Classes	Number of Strains in Each Class																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Lactose	0	4	11	7	2*	2	13	18	15	7	11	7	1	1	1	0	0	0	0	0
Saccharose	3	3	15	9	2*	4	6	3	15	16	8	6	7	0	2	0	0	0	0	1
Salicin	0	5	10	3	4	1*	5	6	10	10	12	13	6	6	5	2	1	1	0	0
Raffinose	2	12	34	16	2	1	0*	2	3	6	0	0	2	0	0	0	0	0	0	0
Mannit	2	18	28	18	7	1*	5	5	5	7	3	0	1	0	0	0	0	0	0	0
Inulin	5	27	16	11	3	1*	2	9	6	8	5	2	3	2	0	0	0	0	0	0

These milk streptococci vary greatly in the number of substances they are able to ferment. This same table (Table 3) shows that the 100 strains fall into 20 groups, based upon the number and

kind of substances fermented. Four strains were able to ferment all of the test media. Fifteen strains fermented five, the largest being the group of 12 strains that fermented all except raffinose. Twenty-seven strains fermented four substances, the largest group in the lot consisting of 15 strains fermenting lactose, saccharose, salicin, and inulin. In the 14 strains which were able to ferment but three of the special media, the largest group consists of nine strains fermenting lactose, saccharose, and salicin; none of the 14 affects raffinose. In the 19 strains which fermented but two substances, chiefly lactose, saccharose, and salicin, inulin is no longer represented, and raffinose by but two (probably aberrant) strains. Fifteen strains fermented but one of the special media, lactose or salicin; most of them—11 strains—fermented salicin. Six strains failed to ferment any of the six test media.

TABLE 3.

MILK STREPTOCOCCI GROUPED IN CLASSES ACCORDING TO THE NUMBER OF SUBSTANCES FERMENTED.

Number (and Percentage) of Strains		Lactose	Saccharose	Salicin	Raffinose	Mannit	Inulin
4	4	+	+	+	+	+	+
15	12	+	+	+	..	+	+
	2	+	+	+	+	..	+
	1	+	+	+	+	+	..
27	15	+	+	+	+
	8	+	+	+	..	+	..
	2	+	+	+	+
	2	+	+	..	+	..	+
14	9	+	+	+
	2	+	..	+	..	+	..
	2	..	+	+	+
	1	+	..	+	+
19	8	+	..	+
	5	+	+
	4	..	+	+
	1	+	+
	1	+	+
15	11	+
	4
6	6
Total.....100		70	66	82	13	27	38

The negative and positive results are both of interest in this connection. The inability of an organism to use one or more of the substances is as definite a character as the fermentative power itself. Thus, we may say that the 12 strains recorded in the second

line of the third table form a group or type of streptococci which are characterized (1) by the power to ferment lactose, saccharose, salicin, mannit, and inulin, and (2) by the inability to ferment raffinose. Similarly, in the fifth line we find a second group of 15 strains characterized (1) by the ability to use lactose, saccharose, salicin, and inulin, and (2) by the inability to use raffinose and mannit. These reaction combinations are much more significant than the mere number or kind of substances fermented. In the 100 milk streptococci tested 20 such combinations or groups (Table 3) occur. They vary greatly in size (one to 15 strains), but the larger groups may be taken as types of the streptococci to be found in milk.

It is possible that the longer period on agar mentioned earlier in connection with the first 31 strains isolated is correlated with the presence of the group fermenting salicin only. These 11 strains grew well in salicin (2.6–6.6 per cent acid). The other 20 strains represent nine of the reaction groups given in the third table. It is therefore probably fair to include the 11 salicin fermenters in this report. An additional reason for including them is that, later, occasional strains about which I felt uncertain with regard to their purity were delayed a similar period and transferred to the special media with the next batch; none of these delayed strains fermented salicin only.

The coagulation of milk (incubated for three days at 37° C.) proved unsatisfactory as a diagnostic character. About half the strains (48 per cent) caused coagulation. There is some indication that the time of year is correlated with these results; e.g., by the middle of April 40 strains had been isolated and but nine of these coagulated milk, while the balance isolated between that time and June 1 contained 39 strains which coagulated milk. In considering milk in connection with the 20 reaction groups (Table 3) we find (1) that the number of these groups is practically doubled, and (2) that the groups are consequently too small to be helpful in indicating types of milk streptococci. Houston feels that coagulation is greatly affected by the temperature used in sterilizing the milk. In view of this unsatisfactory condition of affairs, this qualitative reaction with milk is not considered in the balance of this paper.

As shown by the first table the neutral red (after three days at 37° C. under anaerobic conditions) gave no reduction in 85 per cent of the strains. Reduction was but slight in several of the remaining strains, and since this test has recently been rejected by Houston also as not sufficiently diagnostic, it will not be discussed further in this paper.

Morphological characters, length of chain, degree and regularity of staining, and the presence of occasional abnormal units in a chain do not seem to be correlated with the fermentative powers. One illustration will be sufficient. Eight of the strains used contained chains of 20 cocci or more, even at the last examination from the reincubated slant agar. These eight fall into six different groups of those given in Table 3 with regard to the substances fermented; every substance except mannit was fermented by one or more strains; and a wide range was found in the amount of acid formed: a range of 4.7 per cent in lactose, 5.4 per cent in saccharose, 6.0 per cent in salicin, 2.7 per cent in raffinose, 0.8 per cent in mannit, and 3.2 per cent in inulin.

COMPARISONS WITH EARLIER METHODS AND RESULTS.

The English bacteriologists—Gordon, Andrewes and Horder, and Houston—have done a great deal of work on the fermenting powers of the streptococci. They, however, used the qualitative method, with litmus as the indicator. Houston¹ has recently worked on the streptococci with reference to the possibility of discovering the source of the streptococci found in the London water supply. His reaction groups are indicated by names made ingeniously of the first letters of the test media; e.g., a lamirasacsal organism or type ferments *lactose*, coagulates *milk*, and ferments *raffinose*, *saccharose*, and *salicin*. Since milk is not considered in this paper, it has not been possible to use these convenient names for indicating the reaction groups.

In Houston's² work with milk streptococci, 172 strains, he obtained a higher percentage fermenting lactose and saccharose; his figures for the other substances are lower. The exact percentages

¹ *Fifth Research Report, Metropolitan Water Board, London, 1910.*

² *Report to the London County Council on the Bacteriological Examination of Milk, July 11, 1905.*

follow: lactose (H)¹ 97 per cent, (B)¹ 76 per cent; saccharose (H) 90 per cent, (B) 66 per cent; salicin (H) 60 per cent, (B) 82 per cent; raffinose (H) 19 per cent, (B) 29 per cent; mannit (H) 20 per cent, (B) 27 per cent; and inulin (H) 21 per cent, (B) 38 per cent. When discussing this whole question last summer (1911), Houston said he discarded during isolation all strains which did not ferment lactose. The equine streptococci rarely ferment lactose. He also used the Conradi-Drigalski medium for isolation. These two facts doubtless partly explain these and other differences in our results. Omitting Houston's results with neutral red and milk, and rearranging his reaction groups, we find that he has 16 groups to my 20. In his 16 groups I find but 12 of my groups, and but 74 per cent of my strains.

His figures for equine, bovine, and human fecal streptococci indicate that my milk strains may be of human origin. The human fecal strains may be compared with my milk strains as follows: lactose (H) 76 per cent, (B) 76 per cent; saccharose (H) 86 per cent, (B) 66 per cent; salicin (H) 92 per cent, (B) 82 per cent; raffinose (H) 32 per cent, (B) 29 per cent; mannit (H) 24 per cent, (B) 27 per cent; and inulin (H) 4 per cent, (B) 38 per cent. These compare rather favorably, except for inulin. His largest reaction groups for human strains (when regrouped as above) contain 26, 23, 21, and 11 per cent of his strains; these correspond respectively to 24, 20, 4, and 8 per cent of my milk strains. These results really correspond more nearly to my milk records than to Houston's own milk records. Houston concluded that a certain proportion of milk streptococci are not derived from either human or bovine sources. It is more probable that the qualitative results cannot be so minutely compared. At any rate, while Houston's results with milk differ so much from mine, it is not advisable to lay too much emphasis upon the closer resemblance between his human and my milk strains.

His conclusion that milk streptococci compared with the human strains are more often positive for lactose and inulin, and negative for salicin and raffinose, is but partly supported by my results. Houston's work with bovine, sewage, and water streptococci adds

¹ H indicates Houston's figures; B, my own.

but little, directly or indirectly, in further explanation of my results. In his figures for bovine streptococci, groups containing 75 per cent and 13 per cent correspond to smaller groups of 2 and 21 per cent respectively in my milk strains. In the mixed city sewage where, except for the predominance of equine strains one might expect greater likeness to the results with milk streptococci, both representing the same possible mixed sources, we find still greater differences; the two largest groups, 49 per cent and 44 per cent, are represented by but 1 per cent of the milk strains. The commonest type isolated from London city water fermented (qualitatively, of course) lactose, saccharose, salicin, and raffinose, and is represented by but 4 per cent of my strains.

In 1910 Winslow and Palmer¹ published a comparative study of intestinal streptococci from the horse, the cow, and man. Dextrose, lactose, mannit, and raffinose were used. The results were estimated quantitatively. To test the application of these results to milk streptococci the present study was begun. Dextrose, which seemed to be fermented by practically all the strains, was omitted in my series, and salicin, mannit, and inulin added. For comparison Winslow and Palmer's percentage acidity classes² are reprinted with my milk results (Table 4).

It will be noticed that in the amount of acid formed the milk strains differ greatly, the highest values being remarkably high; in lactose, the highest record was 6.3 per cent, salicin, 7.5 per cent, and in saccharose, 8.4 per cent. The highest for the fecal streptococci (Winslow and Palmer) was 3.6 per cent in lactose. This was reached by but one of their 300 strains, or 0.3 per cent. In the 100 milk strains 3.6 per cent acid was equaled or exceeded by 28 per cent of the strains in lactose, 40 per cent in saccharose, and 56 per cent in salicin. As stated earlier, sugar-free broth was used in making my special media. It averaged 1.6 per cent acid (1.2–2.2 per cent to phenolphthalein) in the controls. This high initial acidity accentuates the difference in the acid values, instead of

¹ *Jour. Infect. Dis.*, 1910, 7, p. 1

² These have been rearranged from Winslow and Palmer's published tables showing the percentage of acid formed by each strain, as a few mistakes were found in their table of percentage acidity classes. To avoid decimals the approximate per cent of strains is given for those obtained from the cow and man.

explaining it. Were it not that some of my records are also lower than those of Winslow and Palmer, this high acid production might be considered to be due to the fact that I used milk as a medium during the isolating period.¹ In this connection it must be remembered that in any milk samples, the streptococci have, of course, grown in milk some time before isolation. In all other respects the method (incubation period, color limit for phenolphthalein reaction, titration in the cold, etc.) was the same as that used by Winslow and Palmer.

TABLE 1.
COMPARATIVE TABLE OF PERCENTAGE ACIDITY GROUPS.

	-1	0	→	+1	2	3	4	5	6	7	8
<i>Lactose</i>											
Horse	66	26	4	2	1	0	1				
Cow	81	16	1	0	0	0	25	9			
Man	17	20	2	4	11	10	20	10	1		
Milk	4	11	7	2	2	15	18	15	7	14	7
<i>Raffinose</i>											
Horse	54	42	1	0	2	1					
Cow	42	30	0	3	7	15	5	1			
Man	20	65	2	1	0	1	2	1			
Milk	2	12	54	16	2	1	0	2	5	6	0
<i>Mannit</i>											
Horse	62	36	0	1	1						
Cow	62	31	2	1	3						
Man	40	28	5	12	10	0	1				
Milk	2	18	28	18	7	1	5	5	5	7	3
<i>Saccharose</i>											
Milk	5	3	15	9	2	4	6	5	15	16	8
<i>Salicin</i>											
Milk	5	10	3	4	1	5	6	10	10	12	15
<i>Inulin</i>											
Milk	5	27	16	11	3	1	2	9	6	8	5

These high records are even more remarkable if the high initial acidity is taken into consideration. Later work will be done with broth controls to see how much the milk affects the acid-forming powers of the streptococci. If the acid results are interpreted as the point at which acidity checked the growth, it is necessary to add the initial acidity to these records; the highest records are then lactose, 8.1 per cent; salicin, 9.0 per cent, and saccharose, 10.1 per cent.

¹ Hilliard and Stowell (*Science*, N.S., 1912, 35, p. 223) have stated that milk streptococci are "much more facultative than throat strains in relation to the temperature at which they are grown."

With these higher acidity values obtained for the milk streptococci occurs a shifting of the intermodal point between the non-acid and the acid-forming groups. Instead of 0.5 adopted by Winslow and Palmer, it is about 1.5 per cent for my milk strains. The results with these fecal streptococci have been recharted here

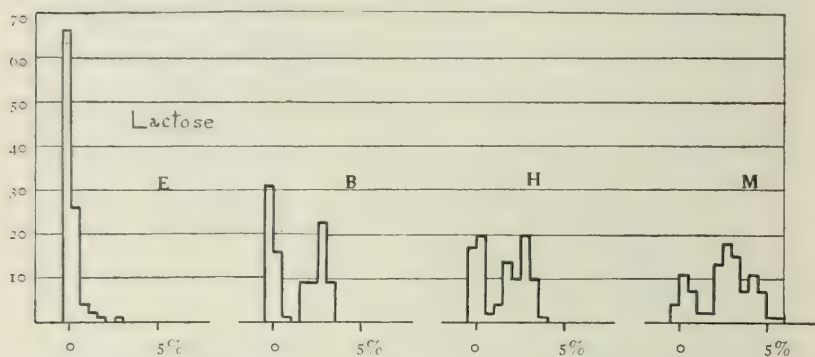


CHART 1.—Acid-producing power of streptococci in lactose. E, equine; B, bovine; H, human; and M, milk.

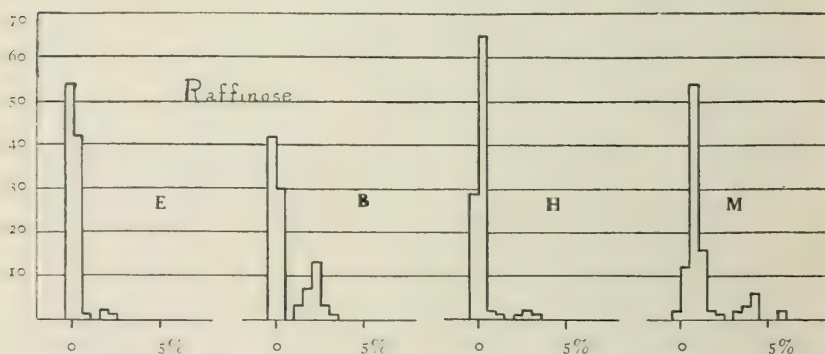


CHART 2.—Acid-producing power of streptococci in raffinose. E, equine; B, bovine; H, human; and M, milk.

for further comparison with my milk streptococci (Charts 1, 2, and 3). In lactose, saccharose, and salicin, for which we have comparable data, it will be noted that the milk charts are more like the human ones in lactose and mannit; in raffinose, the resemblance is nearer the bovine than the human.

Charts are also given for the acid formed in inulin, salicin, and

saccharose (Chart 4). It is unfortunate that similar ones for the fecal streptococci are not available, because (1) inulin has been considered not fermented by streptococci; and (2) salicin and

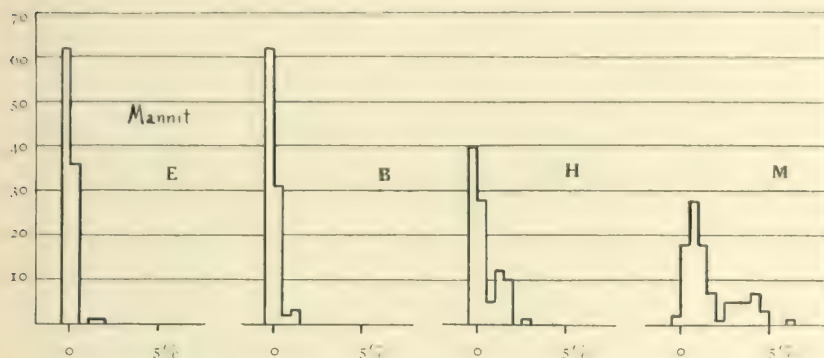


CHART 3.—Acid-producing power of streptococci in mannitol. E, equine; B, bovine; H, human; and M, milk.

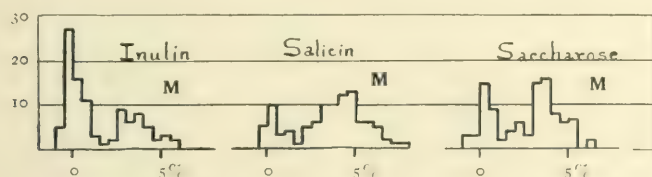


CHART 4.—Acid-producing power of milk streptococci in inulin, salicin, and saccharose. M, milk.

saccharose are much like lactose in the proportion of acid and non-acid strains, and it is possible that they may resemble lactose in diagnostic value.

SUMMARY.

Morphological characters are not correlated with fermentative powers. Milk and neutral red are not sufficiently diagnostic to aid in determining the sources of streptococci. Lactose, saccharose, salicin, raffinose, mannitol, and inulin seem to have significant fermentative reactions. Saccharose, salicin, and inulin should be tested with human, bovine, and equine streptococci. The milk streptococci form a large number of groups when classified with regard to their effect upon the six test substances. The milk

streptococci are characterized by unusually high fermentative powers. The incomplete data at hand indicate that the milk strains are most like the human strains; there is less likeness between the milk and the bovine strains; they show practically no resemblance to the equine strains.

It is proposed to continue this quantitative comparison of fecal (human, bovine, and equine) and milk streptococci in these and other media, in the hope that *complete* quantitative comparisons will give a method of determining the source of streptococcal pollution of milk.

THE CLASSIFICATION OF THE STREPTOCOCCI BY THEIR ACTION UPON CARBOHYDRATES AND RELATED ORGANIC MEDIA.^{*†}

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When Gordon¹ first suggested the systematic use of a number of carbohydrate media in the classification of streptococci it appeared that there was at last some hope of clearing up the relationships of this puzzling group. He studied the action of streptococci upon 35 different organic compounds and finally selected seven as promising the most significant results. These included two disaccharides, lactose and saccharose; one trisaccharide, raffinose; one polysaccharide, inulin; two glucosides, salicin and coniferin; and one alcohol, mannit. He applied these tests, with the clotting of milk and the reduction of neutral red, to 300 cultures from normal saliva,² and to 101 from air.³ Houston examined in the same way 300 strains of streptococci from human feces,⁴ 172 from milk,⁵ and 100 from cow dung.⁶ Andrewes and Horder⁷ made a comparative statistical study of most of these records and of some obtained by themselves in the examination of over 200 pathogenic forms, the entire series including 1,200 strains. They were led to the conclusion that virulence tests had little systematic value on account of the readiness with which this property may be lost or gained. The fermentation reactions, on the other hand, showed themselves remarkably constant, and the difference between long chained and short chained forms appeared on the whole to be of value. When individual strains were considered an almost infinite series of

* Received for publication March 19, 1912.

† Contribution from the Department of Public Health, American Museum of Natural History.

¹ *Rep. of Med. Off. to Local Gov't Bd., Great Britain*, 1902-3, 32, p. 421; *ibid.*, 1903-4, 33, p. 388.

² *Loc. cit.*

³ *Report on an Investigation of the Ventilation of the Debating Chamber of the House of Commons*, 1906.

⁴ *Rep. of Med. Off. to Local Gov't Bd., Great Britain*, 1903-4, 33, p. 472.

⁵ *Report to the London County Council on the Bacteriological Examination of Milk*, July 11, 1905.

⁶ *Rep. of Med. Off. to Local Gov't Bd., Great Britain*, 1904-5, 34, p. 326.

⁷ *Lancet*, 1906, 2, p. 708.

varieties appeared; but when the results were analyzed from the statistical standpoint it was possible to distinguish certain types which were obviously important on account of their numerical frequency. To seven of these types they gave names and characterized them as indicated in Table I.

TABLE I.
ANDREWES AND HORDER'S TYPES OF STREPTOCOCCI.

Name	Milk Clot	Neutral Red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Coniferin	Mannit	Growth on Gelatin at 20° C.	Long Chains	Pathogenesis for Mouse
<i>Str. equinus</i>	+	+	+
" <i>mitis</i>	++	++	++	..	+
" <i>pyogenes</i>	++	++	#	++	+	+
" <i>salivarius</i>	++	#	++	++	#	#	+	+
" <i>anginosus</i>	++	#	++	++	#	+	+
" <i>fecalis</i>	+	+	+	+	+	#	+	+	+	+	..	+
<i>Pneumococcus</i>	#	..	+	+	+	+

Since the publication of Andrewes and Horder's paper, Houston has reported 100 more strains from human feces and 71 from water,¹ and Gordon has reported 155 strains from scarlatinal throats.² In the United States, Winslow and Palmer³ have studied 300 strains from human, bovine, and equine feces, using only four fermentable media, while more recently Miss Broadhurst⁴ has reported tests on 100 milk streptococci and Hilliard⁵ on 65 strains from normal and diseased throats. The results of these observers in some respects conflict with the English work, and even in England Andrewes and Horder's types, after five years, have obtained no general acceptance. The time would seem to be ripe for fresh study of the problem of classifying the streptococci and of the value of Gordon's tests in bringing about its solution.

Two things appear to be necessary if this work is to be carried forward successfully—a uniform and reliable method of determining fermenting power, and a clue to the interpretation of the results obtained. As long as each organic medium is treated as an independent unit, unrelated to any other substance, there can result only a confusing multiplicity of types. It has seemed worth while

¹ *Fifth Report on Research Work, Metropolitan Water Board, London, 1910.*

² *Rep. of Med. Off. to Local Gov't Bd., Great Britain, 1910-11, 40, p. 302.*

³ *Jour. Infect. Dis., 1910, 7, p. 1.*

⁴ *Ibid., 1912, 10, p. 272.*

⁵ *Science, N.S., 1912, 35, p. 223.*

to make a comparative study of the results of the many English and American workers who have dealt with this subject in the hope of attaining these two ends—the recognition of a sound method and the suggestion of a rational basis of relationship between the action of streptococci upon different organic media.

The English results have, without exception, so far as I am aware, been obtained by merely recording positive or negative results as indicated by the reaction of litmus. Gordon¹ describes his method as follows: "Faintly alkaline sugar-free broth was tinted with litmus, charged with the organic substance to be tested, and after inoculation with a given streptococcus, was incubated aerobically for three days at 37°." "Alkaline" presumably means alkaline to litmus since the use of exact titration methods with phenolphthalein as an indicator, almost universal in this country, is rare in England. Thus, when Gordon and Houston report that a streptococcus gives positive results, they mean simply that it changes a medium faintly alkaline to litmus so as to produce reddening.

In the work of Palmer and the writer and that of Miss Broadhurst and of Hilliard, tests for acid production were made by quantitative methods, duplicate tubes being titrated, with phenolphthalein as an indicator. Uninoculated controls were titrated at the same time, and the value obtained subtracted from that found in the inoculated tubes, to give the exact amount produced by the bacteria. When the results of such a study are plotted, they usually show two distinct maxima, one in the neighborhood of the point of no acid formation, the other at a somewhat high acidity. For example, the results obtained by Palmer and the writer for fecal streptococci were as shown in Table 2.

TABLE 2.
ACID PRODUCTION OF FECAL STREPTOCOCCI IN LACTOSE BROTH.

Acid production.....	— .5	0.1	0.6	1.1	1.6	2.1	2.6	3.1	3.6
Percentage normal.....	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	+
Number of strains.....	113	64	7	7	26	19	45	20	1

There is clearly one group centering about the point of no acid production but extending to 0.5 per cent normal on each side

¹ *Rep. of Med. Off. to Local Gov't Bd., Great Britain, 1903-4, 33, p. 388.*

of it, and another group producing 1.5 to 3.5 per cent normal acid, while few strains fall in the region between. According to well known biometrical principles the division between fermenting and non-fermenting strains should be made in this gap and not at any arbitrary point fixed by an indicator. A study of the data published by Palmer and the writer and of the results obtained by Miss Broadhurst and Hilliard, not yet published in full but kindly placed at the writer's disposal, shows that this division line falls for all carbohydrates studied between 0.5 and 2.0 per cent normal and may be placed without serious error at 1.2 per cent normal for all of them.

Litmus is much less sensitive than phenolphthalein, and in ordinary sterile nutrient broth it takes about 0.8 per cent normal hydrochloric acid to change a distinctly blue solution to one that is distinctly red. If this relation were general the litmus test ought to give somewhere near the same result as the titration test (using 1.2 per cent normal increase in acidity as positive), and this is why the two methods roughly correspond. The difference between alkaline and acid litmus broth will be more or less sharp, however, according to the judgment of the observer, and the litmus method must be less accurate than the titration method. Furthermore, the presence of some constant difference seems to be indicated by the comparison of English and American results, tabulated in Table 3.

TABLE 3.
COMPARISON OF RESULTS OBTAINED BY ENGLISH AND AMERICAN OBSERVERS.
Percentage of Fermenting Strains.

SOURCE OF STREPTOCOCCI	THROAT		HUMAN FECES		BOVINE FECES		MILK	
Observer	Hilliard	Gordon	Winslow Palmer	Houston	Winslow Palmer	Houston	Broadhurst	Houston
		A B						
Lactose.....	63	81 97	62	100	52	100	87	100
Saccharose.....	38	100 100	..	85	..	94	69	99
Salicin.....		40 62	..	93	..	95	75	72
Inulin.....		13 5	33	..
Mannit.....	0	0 2	28	31	6	0	31	15
Raffinose.....	4	48 28	6	26	28	80	11	62

A = Normal throat. B = Scarlet fever throat.

With three exceptions, the English results are higher, usually very much higher, and it seems reasonable to attribute this to the

difference in technic rather than to any national peculiarities in the streptococci tested. Of course the choice of the titration method (aside from its obviously greater exactness) rests on the assumption that the biometrical method of classification is a valid one and that the dividing line between fermenting and non-fermenting strains should be made at the intermodal point of the frequency curve. If this be granted, the American method appears to be accurate; and if it be accurate the English method appears to yield results which are too high. In view of this constant difference and in view of the obvious inexactness of the litmus test it seems that systematic studies of the fermentative relations of the streptococci should be carried out by titration with phenolphthalein as an indicator. Media may be made up from meat extract (each batch checked by controls inoculated with *B. coli*) and adjusted to an initial reaction between neutral and 0.5 per cent normal acid. Dextrose, lactose, saccharose, salicin, inulin, mannit, and raffinose should all be used for diagnosis, for the present at least, and titration may be made after cultivation for three days at 37° C. When studied in this way, cultures producing over 1.2 per cent normal acidity more than that initially recorded may be considered positive.

The second aim of the present study was to examine the relations between the fermentability of the different organic media. For this purpose, all available results obtained by the titration method have been compared. These include 202 strains of streptococci from human and bovine feces studied by Palmer and the writer, 101 strains from milk studied by Miss Broadhurst, 65 strains from normal and diseased throats studied by Hilliard, and 17 from various sources studied by T. D. Ballinger at the American Museum under the general direction of the writer, making 385 in all. The detailed results of the last two observers, not yet published in full, have been courteously furnished for the purpose of this analysis.

The relative frequency with which the various media are attacked is indicated for the entire series in Table 4.

It appears at once from this table that dextrose, lactose, salicin, and saccharose are readily fermentable, half or three-quarters of all strains giving positive results, while mannit, inulin, and raffinose

are much less readily attacked. The data are, however, not wholly comparable. Salicin tests, for example, were made only by Miss Broadhurst and Ballinger working almost wholly with milk strains, which are always high fermenters; hence the salicin percentage

TABLE 4.
FERMENTABILITY OF VARIOUS ORGANIC MEDIA BY THE STREPTOCOCCI.

	NUMBER OF STRAINS			PERCENTAGE POSITIVE RESULTS
	Fermenting	Failing to Ferment	Not Tested	
Dextrose	244	53	88	82
Lactose	240	130	..	65
Salicin	88	30	267	75
Saccharose	101	82	202	55
Inulin	38	80	207	32
Mannit	60	310	..	18
Raffinose	40	330	..	12

is higher than it should be relatively, while one-third of all the strains tested in saccharose were Hilliard's throat strains, which are frequently low fermenters. The only way to get the actual relative availability of the different media is to study the individual correlations; and these have been worked out for all the tests made in Table 5.

TABLE 5.
CORRELATIONS OF DIFFERENT ORGANIC MEDIA IN REGARD TO FERMENTABILITY.
Number of Strains in Each Class.

		Lactose		Salicin		Saccharose		Mannit		Inulin		Raffinose	
		+	-	+	-	+	-	+	-	+	-	+	-
Dextrose	+	162	82	20	8	42	47	42	202	6	22	25	219
	-	18	35	0	2	0	6	0	53	0	2	10	43
Lactose	+	74	24	92	47	66	183	45	53	50	199
	-	14	6	9	35	3	133	1	19	3	133
Salicin	+	63	26	41	47	35	53	9	79
	-	12	17	2	28	5	25	6	24
Saccharose	+	38	63	37	38	15	86
	-	5	77	1	42	1	81
Mannit	+	20	23	9	60
	-	18	57	37	279
Inulin	+	9	29
	-	6	74

The substances studied are here arranged in what appears to be on the whole their order of relative availability. If any member of

the series is fermented, the chances are that those ahead of it will be fermented also. If any member is not attacked, the chances are that those behind it will not be attacked either. For example, a positive result with dextrose does not imply a positive result with any other substance, but a negative result with dextrose does imply a probably negative result with all other substances. Thus of the strains failing to act on dextrose, two-thirds did not ferment lactose, four-fifths did not ferment raffinose, and none attacked any other of the substances tested. Of the strains fermenting inulin, all attacked dextrose, all but one lactose and saccharose, and 35 out of 40 attacked salicin, though only half fermented mannit. There are exceptions, of course, as would be expected, since the variability of organisms under the conditions of such experiments are always considerable. The exceptions to the general rule are indicated by the figures in the lower left hand corner of each correlation table in heavy type. In the cases of lactose and salicin, salicin and saccharose, mannit and inulin, and mannit and raffinose, these exceptions are considerable, and all these are examples of substances lying close to each other in the series. The general trend of the figures may perhaps be brought out by adding together the heavy type figures (giving a total of all cases in which a substance was attacked while one higher in the series was not) and comparing with the corresponding total for the upper right hand figure in each correlation table (total of all cases in which a substance was attacked while one lower in the series was not). The total of the lower left hand figures is 151; of the upper right hand figures 1,590. The relations are perhaps better shown in Table 6.

Thus if any given substance is fermented, only between 23 and 42 per cent of the substances lower in the scale are acted upon, while between 85 and 95 per cent of those higher in the scale are fermented (excepting only in the case of raffinose, which is somewhat aberrant). If a substance is not fermented, only from 20 to 49 per cent of those preceding it in the scale are not acted on, while 80 to 97 per cent of those following it fail to show a reaction.

I have tried to derive a mathematical expression for these relations but so far without success. It seems clear, however, that the substances tested do stand to each other in a definite order of

availability. Howe,¹ has described the same phenomenon in the fermentative power of the colon bacillus and its allies, and suggested for it the excellent name of "metabolic gradient."

TABLE 6.
RELATION BETWEEN FERMENTABILITY OF DIFFERENT ORGANIC MEDIA.

	GIVEN SUBSTANCE			
	+		-	
	Percentage of		Percentage of	
	Subsequent Substances +	Preceding Substances +	Subsequent Substances -	Preceding Substances -
Dextrose...	34	..	83	..
Lactose...	30	81	92	30
Salicin...	42	87	80	20
Saccharose...	32	90	97	33
Mannit...	26	95	86	37
Inulin...	23	85	92	43
Raffinose...	..	65	..	49

The order of availability, so far as the streptococci are concerned, corresponds closely to what might be expected from the chemical composition of the substances concerned. First comes the monosaccharide, dextrose, then the disaccharides, lactose and saccharose, and the glucoside, salicin. The latter it will be remembered is an ethereal derivative ($C_{13}H_{18}O_7$) which is easily broken up by acids, alkalies, or enzymes to yield a monosaccharide. Considerably lower in the scale stand the hexatomic alcohol mannit ($C_6H_{14}O_6$) and the starch-like body, inulin. Least available of all is the trisaccharide, raffinose, but this body does not fit into the metabolic gradient as closely as the other substances. In Table 5 it appears that a considerable proportion of strains which ferment raffinose are negative in dextrose, salicin, mannit, and inulin.

It is interesting to note that the order of availability of the various substances is not the same for the colon bacilli and the streptococci. Dextrose is most generally attacked in each case and lactose comes next. Among the streptococci, saccharose is almost as easily attacked as lactose, while raffinose is rarely fermented. That is, the size of the molecule appears to be the main factor involved. With colon bacilli, on the other hand, the lactose fermenters are divided into two groups of about equal size by

¹ *Science*, N.S., 1912, 35, p. 225.

the saccharose test; and it has been shown by Walker and the writer,¹ and others that when saccharose is attacked raffinose is usually fermented also. With these bacilli it is not the size of the molecule which is significant but its configuration, the sugars with an aldehyde grouping being easily attacked, while the ketonic sugars (saccharose and raffinose) are less commonly fermented, whether they are disaccharides or trisaccharides.

It seems possible that the conception of the metabolic gradient may materially simplify the classification of bacteria by their fermentative reactions. For the present as large a series of substances as possible should be tested in order to confirm the conclusions here suggested. Ultimately, however, it may be possible, for example, to recognize a group of streptococci fermenting monosaccharides only, and another group attacking disaccharides also (using as a test one disaccharide only and leaving out perhaps saccharose and salicin), while a third group ferments the trisaccharides also. With the colon group, on the other hand, saccharose is of prime importance as an index of the power to attack ketonic sugars, while additional tests with raffinose add little to our knowledge.

The results already attained by biometric studies, using the quantitative titration method, seem sufficiently promising to warrant carrying them further. Thus the work of Palmer and the writer suggested that mannit-fermenting streptococci are particularly characteristic of human feces and raffinose-fermenters of the feces of cattle. Hilliard finds that throat streptococci do not usually attack any substance more complex than the disaccharides and that milk streptococci can be generally distinguished from throat strains by the amount of acid formed at 20°. Broadhurst has shown that although milk streptococci are more closely related to human than to bovine strains, they have an unusually wide range of fermentative power and produce a very high actual acidity. All these more or less preliminary results suggest that the biometric study of fermentative powers may not only throw light on the systematic relationship of the cocci but may yield results of practical sanitary importance.

¹ *Science*, N.S., 1907, 26, p. 797.

EXPERIMENTAL THERAPY OF ROCKY MOUNTAIN SPOTTED FEVER.*

THE PREVENTIVE AND CURATIVE ACTION OF A SERUM FOR SPOTTED FEVER, AND THE INEFFICIENCY OF SODIUM CACODYLATE AS A CURATIVE AGENT FOR THIS DISEASE IN GUINEA-PIGS.

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In a preliminary note on the "Production and Concentration of a Serum for Rocky Mountain Spotted Fever"¹ we reported the preparation of a serum for Rocky Mountain spotted fever and its potency, determined by tests on guinea-pigs. Additional experiments have confirmed our previous results and have shown that the serum has a limited curative effect on guinea-pigs inoculated with spotted fever virus, if given during early stages of the disease. A series of experiments upon the efficacy of sodium cacodylate as a curative agent has also been completed, the results of which are reported in this paper.

SPOTTED FEVER SERUM.²

In the spring of 1907 Ricketts and one of us (H.), while working on spotted fever problems in Missoula, Mont., inoculated two horses with spotted fever virus. One of the horses was inoculated subcutaneously with 65 c.c. obtained from infected guinea-pigs, the other horse with 80 c.c. from a monkey. The first horse had a temperature of 105.6° on the third day after inoculation. The temperature fell rapidly and no tests were made of the infectiousness of the blood or of the protective power of the serum. Another injection of 60 c.c. blood from a spotted fever patient and an infected monkey gave no reaction. The second horse did not

* Received for publication April 8, 1912.

¹ *Jour. Am. M. Ass.*, 1911, 57, p. 198.

² Early experiments with horses, carried on by the late Dr. Ricketts in conjunction with us, have never been published in full. Brief reference is made to the work with two horses in Missoula in 1907 in *Jour. Am. M. Ass.*, 1907, 49, p. 24. Other results of his work mentioned in this paper are taken from Dr. Ricketts' original notes.

react. The following winter (1908) we inoculated two horses. The first one of these received 110 c.c. guinea-pig virus intra-peritoneally. Some blood was drawn before injection to test the natural protective power of horse's blood. The results were negative. The maximum temperature was reached on the third day (103.6°). Several tests of the infectivity of the blood for guinea-pigs during the course of the fever were made. A positive result was obtained only from the blood obtained on the day of highest temperature and the result confirmed by passage from the first guinea-pig to a second one. Both had typical spotted fever determined by the temperature curve, external lesions, and autopsies. The protective power of serum drawn after subsidence of fever was 1 c.c. The second horse inoculated did not react. The first horse was bled to death and the serum put up in vials containing about 20 c.c. each and sent to the Bitterroot Valley in Montana for use. No systematic reports were received of its value in the treatment of the disease. Two or three injections with this serum caused severe rashes, which vanished after a day or two, and probably were due to the well-known effect of fresh horse serum.

During the same year (1908) Ricketts, assisted by one of us (M.), inoculated two horses. One received 80 c.c. guinea-pig virus, the other 85 c.c. guinea-pig virus and an emulsion of the livers and spleens of the same guinea-pigs. Results in both cases were negative.

We resumed this work in the early spring of 1911 with financial assistance of the counties of Ravalli and Missoula in western Montana. Two horses were purchased and injected with virus. A third horse, also injected, did not respond. The first horse received 220 c.c. guinea-pig virus. The virus was obtained by injecting guinea-pigs and bleeding them to death on the second day of the fever. The defibrinated blood was injected. Previous experiments were not extensive enough to show preference for any method of injection. For this reason we concluded to divide the virus into two equal parts and inject one part into the jugular vein in the neck, and the other part subcutaneously back of the shoulder. About three weeks later this horse received an injection of 460 c.c. virus, also by intravenous and subcutaneous

injections. The second horse received but one injection of 250 c.c. guinea-pig virus.

The temperatures were taken twice daily and are recorded in Table 1. The maximum temperature of horse 1 was 103.5° on the fourth day after injection, of horse 2 on the third day (103.4°). After horse 1 had received the second injection no appreciable fever developed, excepting for an hour or two shortly after injection. This was probably due to anaphylactic shock, as the horse was covered with a profuse perspiration and trembled violently.

TABLE 1.
TEMPERATURES OF HORSES INJECTED WITH SPOTTED FEVER VIRUS.

HORSE	TEMPERATURES AFTER DAYS											
	0		1		2		3		4		5	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
1		102.6	101.2	99.9	99.8	101.6	100.6	102.0	102.8	103.5	102.1	102.5
2		100.8	98.9	98.8	101.0	101.0	102.4	103.4	103.4	102.0	101.0	100.8
1. After 2d injection.....		100.8	100.5	101.8	100.8	100.2	101.5	101.2	101.2	101.2	101.0	100.8

TABLE 1.—Continued.

HORSE	TEMPERATURES AFTER DAYS											
	6		7		8		9		10		11	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
1	101.8	103.0	101.8	102.4	101.0	101.0	99.6	99.8	99.6	99.6	99.4	99.8
2	102.0	99.4	100.4	100.5	99.2	99.8	98.8	99.2	99.2	99.4	99.2	99.4
1. After 2d injection....	100.2	100.5	99.8	99.6	99.8	99.8	99.6	98.8	98.8	99.2	99.6	99.8

Both horses were bled every day during the period of temperature and the defibrinated blood injected into guinea-pigs in doses of 1 c.c. and 5 c.c. A positive result was obtained only the day of the highest temperature in horse 1. The result was confirmed by passage from the first guinea-pig into a second one. Curiously the 1 c.c. injection only was positive. The 5 c.c. injection showed no result. This observation was made by Ricketts also and he explained the phenomenon on the ground of the presence of immune

bodies in horse's blood. The mild course of the fever in the horse would tend to confirm this theory. Through oversight no blood was drawn from horse 2 on the day of highest temperature, and all tests on guinea-pigs were negative.

After the fever had subsided blood was drawn every second day and the serum tested for protective power. At first the results were negative, but gradually protection seemed to develop and reached the highest efficiency after 12 days. These tests were made by injecting 1 c.c. virus in all cases and varying amounts of serum. The virus and serum were injected separately into the peritoneal cavity. We used guinea-pigs weighing approximately 250 gms. It is true that according to Ricketts' statements 0.1 c.c. virus is always infectious, and 0.05 c.c. in most cases, and 0.01 c.c. in many cases. However, we aimed to make the tests as rigorous as possible and used 1 c.c. The virus and serum were never mixed before injection, to eliminate a possible reduction of virulence by the action of the serum. As controls we injected in all experiments reported a guinea-pig with virus only and one with virus and 5 c.c. normal horse serum. The results were considered positive if the guinea-pig survived without having shown marked rise in temperature. If the guinea-pigs died the diagnosis of spotted fever was based on temperature curves, external lesions, and the findings after autopsies.

In order not to burden the tables with too many figures we have recorded only one typical temperature curve of guinea-pigs used for controls and only one where normal horse serum was used. We have tabulated these in Table 2 together with some selected temperature protocols, representing the limits of potency of the sera. The serum was diluted with physiological salt solution to 4 c.c. in one series of experiments. For some unexplained reason the guinea-pigs endured injections of undiluted serum better than of diluted serum. Those injected with diluted serum seemed to succumb to the influence in many instances, while those injected with undiluted serum showed no untoward effect.

The table shows that the native serum of horse 1 drawn after 12 days protected in doses of 5 c.c., but not if only 1 c.c. was given. We did not determine the amount between 1 and 5 c.c. which

TABLE 2.
TEST OF POTENCY OF SPOTTED FEVER SERA AGAINST 1 C.C. VIRUS.

KIND OF SERUM	AMOUNT	No. of GUINEA-PIG	TEMPERATURE AFTER DAYS															REMARKS	
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14		15
Horse 1. Serum drawn 12 days after temperature subsided	0.1 c.c.	1	102.8	102.8	102.4	102.6	105.2	104.6	106.0	106.0	106.2	106.0	103.8	103.4	103.0	102.6	102.4	102.5	No protection. Typical course of spotted fever
	0.5 c.c.	2	102.6	102.6	102.6	103.0	103.6	102.6	105.4	106.2	106.0	105.2	105.4	105.4	died	Died of spotted fever after 12 days
	1.0 c.c.	3	102.8	103.4	103.6	104.8	105.2	106.0	106.2	106.0	105.4	105.4	105.4	105.2	died	Died of spotted fever after 12 days
Control, virus only Virus and normal horse serum...	5.0 c.c.	4	102.0	102.6	102.8	103.0	103.0	103.6	103.0	103.0	102.6	102.6	102.8	102.6	102.8	102.6	102.8	102.6	Protected
	5	102.8	102.8	103.6	104.6	105.2	106.2	106.4	106.0	105.2	died	Died of spotted fever after 6 days
	5.0 c.c.	6	102.2	102.4	102.4	103.2	105.0	105.2	106.4	106.2	105.8	104.0	died	Died of spotted fever after 6 days
	7	102.4	102.0	102.0	103.2	102.8	102.2	103.6	103.8	104.0	105.2	106.0	105.0	104.0	103.0	died	Fever delayed, died of spotted fever after 14 days
Horse 2. Serum drawn 12 days after 2d infection	0.125 c.c.	8	102.6	103.2	102.2	103.2	102.8	102.6	103.0	103.2	103.6	104.8	105.0	105.2	105.2	104.0	102.2	101.8	Mild course of fever, recovered
	0.25 c.c.	9	102.8	103.0	102.2	103.0	102.8	102.4	103.2	102.8	102.6	102.4	102.0	102.8	102.0	103.0	102.8	102.0	Complete protection
Horse 2. Serum drawn 12 days after 2d infection	0.5 c.c.	10	103.0	102.2	103.2	103.0	103.2	103.6	102.6	103.0	103.6	104.2	105.0	105.2	105.2	104.0	103.0	102.8	Mild fever, probable protection
	0.25 c.c.	11	102.8	103.0	102.2	103.0	102.8	102.4	103.2	102.8	102.0	103.0	102.6	103.8	103.0	104.0	102.2	102.6	Complete protection
	0.75 c.c.	12	102.6	102.6	102.0	102.4	102.4	102.0	103.2	103.0	102.6	102.6	102.4	104.0	103.6	103.8	102.6	102.6	Complete protection

would protect. The serum from horse 2 protected if 0.5 c.c. were given. If 0.25 c.c. were given the fever ran a mild course and the guinea-pig recovered. The serum of horse 1 drawn after the second injection protected definitely if 0.5 c.c. were given, while doses of 0.25 and 0.125 c.c. seemed to cause the fever to take a mild course.

Since antidiphtheric serum has been concentrated successfully during recent years we thought that possibly spotted fever serum might be concentrated by the same method. The experiment was successful, as is shown in Table 3. The serum from horse 1 after the second injection of virus was concentrated and then protected if 0.05 c.c. were given. The concentrated serum of horse 2 protected in doses of 0.01.

The favorable results of concentrating the serum show that the antibodies to spotted fever in horse's blood are united with the pseudo-globulin fraction, the same as in diphtheria and tetanus antitoxin. The decided gain in potency resulting from the second injection given to horse 1, coupled with the possibility of increasing the potency by concentration, seem to indicate that by giving frequent injections of virus according to a similar plan now carried on in the preparation of diphtheria antitoxin, highly potent sera may be obtained. We expect to test this possibility as soon as opportunity offers.

The concentrated serum of horse 2 was put up in vials containing about 25 c.c. each and sent to Missoula for use during the season of spotted fever. The serum was, of course, sterilized by passing through a Berkefeld filter, and a safety test made by injecting 5 c.c. into the peritoneal cavity of a guinea-pig. It seems that not sufficient interest was taken in trying this serum, so that its efficiency for human beings remains problematical. The eradication of spotted fever in the Bitter Root Valley is a matter of vital importance, not only locally, but also because of the likelihood of the fever spreading throughout the neighboring states as the population increases. By systematic attempts at eradicating the ticks and the lower animals serving as hosts for ticks much may be accomplished. These methods, however, will take considerable time before results can be expected, and it would seem that a serum of prophylactic and possibly curative value might afford immediate

TABLE 3.
TESTS OF POTENCY OF CONCENTRATED SPOTTED FEVER SERA AGAINST 1 C.C. VIRUS.

KIND OF SERUM	AMOUNT	No. OF GUINEA- PIG	TEMPERATURE AFTER DAYS															REMARKS	
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14		15
Horse 1. Concentrated serum after 24 injection	0.01 c.c.	13	102.6	102.6	102.6	102.6	103.0	104.6	104.6	105.0	105.0	104.2	104.6	104.4	104.6	104.0	103.0	102.6	Partial protection
	0.03 c.c.	14	103.4	102.4	102.0	101.2	103.0	105.2	105.4	105.4	104.6	104.8	104.0	103.6	104.0	103.8	102.8	102.4	Partial protection
	0.08 c.c.	15	103.0	102.0	102.0	101.2	103.2	102.6	102.6	103.0	103.2	103.0	103.2	103.0	102.4	102.6	102.0	102.4	Complete protection
Horse 2. Concentrated serum.....	0.01 c.c.	16	102.6	103.0	102.8	102.4	102.1	102.2	102.8	103.0	103.4	103.0	102.6	102.0	102.0	102.2	102.0	102.2	Complete protection
Horse 2. Concentrated serum after 12 mos..	0.08 c.c.	22	103.0	102.6	102.6	102.8	103.6	105.0	104.3	104.0	103.8	103.6	102.4	102.6	Complete protection

relief. Systematic administration as prophylactic in cases of tick bites in infected regions and as curative agent during early stages of the disease is the course indicated to determine this question.

In order to confirm all potency tests the guinea-pigs surviving were given immunity tests by injecting 1 c.c. virus. The results appear in Table 4. All but number 4 did not take the disease and were therefore immune. Whether this is passive or active immunity is difficult to decide. We are inclined to think it is passive immunity. At any rate immunity lasted for at least four weeks, as is evident from the fact that a second immunity test in guinea-pigs 8, 9, 10, 11, and 12 was positive, inasmuch as the guinea-pigs did not take the disease from a second injection of 1 c.c. virus. In guinea-pig 4 the immunity was probably of short duration, since it ran a typical, although prolonged, course of spotted fever and died on the 12th day.

To determine the amount of deterioration of protective value of concentrated serum from horse 2, a series of tests was made after the lapse of 12 months, during which time it was kept in an ice chest. The former protective dose of 0.01 was reduced to 0.08, and partial protection afforded with 0.05. The same serum protected in subcutaneous injections in a dose of 0.1 c.c. Smaller amounts were not tested.

The next series of experiments were conducted with the object of determining whether the serum can influence the course of the disease, if given at various periods after injection of the virus. We inoculated five guinea-pigs with 1 c.c. virus and then injected 1 c.c. concentrated serum on different days. Guinea-pig 17 received serum on the first day after injection of virus, guinea-pig 18 on the second day, guinea-pig 19 on the third day, guinea-pig 20 on the fourth day, and guinea-pig 21 on the fifth day. The results appear in Table 5. Guinea-pigs 17 and 18 had but slight changes in temperature and recovered completely. The serum evidently took effect before the first appearance of temperature. Guinea-pig 19 had a temperature of 105.2° the day the serum was given. The temperature was down to 103° the next day, and no important changes took place after that and the animal recovered. Guinea-pigs 20 and 21 received serum after temperature was high for one

TABLE 4.
IMMUNITY TESTS ON GUINEA-PIGS, WHICH SURVIVED IN TABLES 2 AND 3.

No. of Guinea-Pig	TEMPERATURE AFTER DAYS															REMARKS		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14		15	
1.....	102.8	102.0	103.0	0	102.0	103.1	103.4	103.3	2	103.0	102.0	102.6	103.0	102.6	102.8	102.6	Immune, second immunity test gave the same result	
4.....	102.0	102.8	103.2	2	103.0	103.0	103.5	103.0	100.0	105.0	105.0	105.2	died		
8.....	103.0	103.8	103.1	0	103.0	103.0	103.3	103.0	102.8	102.6	102.8	103.0	103.4	102.8	103.0	103.2	Immune, second immunity test gave the same result	
9.....	103.0	103.8	103.4	0	103.0	103.0	103.3	103.0	103.0	104.2	103.6	103.2	103.8	103.0	103.2	103.2		
10.....	103.0	103.0	103.8	103.0	103.4	103.0	103.0	102.6	102.6	102.8	103.0	103.8	103.0	103.2	102.8	102.8	Immune, second immunity test gave the same result	
11.....	103.2	104.0	103.8	103.0	103.4	103.0	103.0	103.0	103.0	2	104.2	103.8	103.8	103.0	103.2	103.0		
12.....	103.2	104.2	103.8	103.4	103.0	103.6	103.0	102.8	103.0	104.0	104.1	104.8	102.6	102.4	102.4	103.0	Immune	
13.....	103.4	103.0	103.8	103.0	103.4	103.0	102.6	102.6	102.8	103.0	103.6	102.8	102.8	103.0	103.0	102.8		
14.....	103.0	103.0	103.8	103.2	103.0	103.6	103.0	102.4	103.0	1	103.6	103.2	103.0	102.8	102.4	102.6	Immune	
15.....	103.0	102.0	103.0	103.0	103.4	103.0	2	102.4	102.6	103.0	103.0	102.8	102.6	102.4	102.6	102.6		
16.....	102.0	102.0	102.0	103.0	0	101.0	102.0	0	101.5	102.0	102.0	103.2	102.4	101.0	102.0	102.2	101.8	Immune
17.....	102.0	102.0	102.0	102.0	102.0	102.0	102.0	102.0	102.0	102.0	102.0	102.0	102.0	102.0	102.0	102.0	102.0	

TABLE 5.
CURATIVE VALUE OF CONCENTRATED SERUM FROM HORSE 1, AFTER SECOND INJECTION.

DAYS AFTER WHICH SERUM WAS INJECTED	NO. OF GUINEA-PIG	TEMPERATURE AFTER DAYS															REMARKS	
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14		15
1.....	17	102.6	103.2	102.0	101.0	100.0	103.0	103.4	103.2	103.0	103.0	103.0	103.0	103.0	103.0	103.2	102.6	Complete protection
2.....	18	102.4	102.4	102.5	103.0	104.0	103.0	103.0	103.4	103.8	103.0	104.0	103.0	103.0	102.6	102.2	102.0	Complete protection
3.....	19	102.0	102.0	102.4	105.2	103.0	103.0	103.0	103.4	103.2	103.0	104.0	103.0	103.0	102.5	102.5	102.6	Complete protection
4.....	20	102.0	103.0	103.0	104.0	106.2	105.2	105.2	105.2	105.2	105.2	105.2	104.2	died	Died of spotted fever on 11th day
5.....	21	102.4	102.8	103.2	104.2	105.0	105.0	105.0	106.0	105.8	105.2	died	Died of spotted fever on 9th day

and two days respectively. Both animals died of spotted fever. Control animals were inoculated as usual. These experiments seem to show that the serum may have value as a prophylactic measure and may even have curative effect. It must, however, be given early in the disease. After temperature has persisted for some time the serum seems to be ineffective. One c.c. in a guinea-pig corresponds to about 300 c.c. in a man of 150 pounds weight. The serum should be given in large doses and the injection repeated at frequent intervals. No harm can result from large doses, as has been demonstrated many times by the administration of several 100,000 units of diphtheria antitoxin. We did not determine whether smaller amounts than 1 c.c. serum have prophylactic effects on guinea-pigs.

We intend to take up experiments with the preparation and effect of serum from immunized horses when opportunity offers. Our success in increasing the potency materially by a second injection encourages the belief that repeated injections of virus will produce a powerful serum, the potency of which can be increased materially by concentration.

EXPERIMENTS WITH CACODYLATE OF SODIUM.

Recently claim has been made that sodium cacodylate is effective in the cure of spotted fever. Ricketts and Matthews some time ago carried on preliminary experiments, which were not published because the results were not conclusive.¹ We therefore undertook a series of experiments with different amounts of this drug. Ricketts and Matthews determined that 8 mgm. was the largest amount that guinea-pigs will stand without untoward effect. We confined our tests to doses varying from 2 mgm. to 8 mgm. The injections were given subcutaneously. Twenty-six guinea-pigs were inoculated with 1 c.c. virus each. On the third day six guinea-pigs received 2 mgm. sodium cacodylate dissolved in sterile water. Another set of six guinea-pigs received 4 mgm., a third set 6 mgm., and a fourth set 8 mgm. The remaining two guinea-pigs were reserved as controls. The injection of cacodylate of sodium was repeated every day until death ensued. All these guinea-pigs, including the controls, died of spotted fever in seven to nine days.

¹ They also tested the efficiency of atoxyl, spirarzyn, and quinine sulphate with negative results. We confined our experiments, therefore, to sodium cacodylate.

No difference could be detected between the guinea-pigs which had been treated with cacodylate of sodium and the two controls. The temperature curve was typical of spotted fever in all cases and the diagnosis was confirmed by autopsies. We feel justified in the conclusion that sodium cacodylate has no effect on the course of spotted fever in guinea-pigs.

CONCLUSIONS.

1. Horses are susceptible to spotted fever if the virus of guinea-pigs is injected subcutaneously and intravenously. The fever usually takes a mild course and the temperature is not exceedingly high. Normal temperature appears again after seven to nine days. It would be interesting to allow infected ticks to bite horses and determine whether the disease can be communicated that way.

2. The serum from horses recovered from spotted fever has protective value. The potency is largest after about 12 days from the time of the reappearance of normal temperature.

3. Repeated injection of spotted fever virus increases the potency of the serum materially, but does not produce a second attack of spotted fever.

4. The serum can be concentrated by the method practiced in concentrating diphtheria antitoxin. The gain in potency may be 10 times the original value.

5. The pseudoglobulin fraction of blood serum contains the bulk of antibodies in immunized horses.

6. Assuming 0.1 c.c. of guinea-pig virus to be the smallest amount which will invariably produce spotted fever in guinea-pigs, 1 c.c. of a serum was protective against 1,000 doses.

7. Guinea-pigs injected with spotted fever virus and immune horse serum separately into the peritoneal cavity acquire an immunity lasting for at least four weeks.

8. One c.c. immune horse serum protects guinea-pigs injected with spotted fever virus up to, and including, the first day of high temperature. If serum is given later there is no protection.

9. Treatment of guinea-pigs, injected with spotted fever virus, with sodium cacodylate and repeated every day, commencing with the first appearance of temperature, has no effect on the course of the disease.

EXPERIMENTS ON DISINFECTION OF WATER WITH ULTRA-VIOLET LIGHT, WITH A DISCUSSION OF THE LAWS OF DISINFECTION.*†

MAURICE R. SCHARFF.[‡]

INTRODUCTION.

The value of sunlight in human dwellings as an aid to healthy living has been recognized from involuntary human experience from time immemorial. The actual germicidal effect of light has been a subject of study ever since 1877, when Downes and Blunt,² in a classic series of experiments, demonstrated that light inhibited bacterial growth, and that the blue end of the solar spectrum was the more efficient for this purpose. A host of investigators studied the phenomenon in the eighties and nineties, some turning to the electric arc as a source of bacterial light, and a group, headed by Finsen, made the first application of the phenomenon in the treatment of certain forms of tuberculosis with the Finsen lamp. It was not until within a few years, however, that the invention of the quartz tube mercury arc lamp, and an awakened interest in water disinfection gave impetus to the study of water disinfection with ultra-violet light.

Curiously enough, the first work of this kind appears to have been done on milk. An anonymous communication to *Die Milchzeitung* (1909) states that Privatdozent Max Seiffert invented an apparatus for sterilizing milk with ultra-violet light in 1901. And Billon-Daguerre³ (1909) in a communication to the Académie des Sciences, dated January 7, 1907, claims priority in the invention of a similar method.

The first published account of experiments in disinfection of water with ultra-violet light are by Courmont and Nogier⁴ (1909). They have been closely followed by many others, including Billon-Daguerre⁵ (1909), Henri, Helbronner, and de Reckling-

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² *Proc. Roy. Soc.*, 1877, 26, p. 488; 1878-79, 28, p. 199; 1886, 40, p. 14.

³ *Compt. rend. Acad. d. Sci.*, 1909, 148, p. 542.

⁴ *Ibid.*, 147, p. 523.

⁵ *Ibid.*, 149, p. 810; 1910, 150, p. 479.

hauser¹ (1910), Gabriel-Vallet² (1910), Urbain, Scal, and Feige³ (1910), Cernovodeanu and Henri⁴ (1910), and Grimm and Weldert⁵ (1911).

These observers have reported disinfection or sterilization of water with current consumptions varying from 75 to 900 kilowatt hours per million gallons. Others have studied the chemical effects of light on air and water, and still others have determined spectrographically which light waves are most effective in this respect. There has been a notable lack, however, of any satisfactory quantitative measure of the effect of varying such factors as distance, absorption, etc., and hence no working hypothesis suitable for the rational design of an apparatus for disinfecting water. It was with a view to determining whether or not these deficiencies could be supplied that the studies on which this paper is based were undertaken.

ON THE STANDARDIZATION OF DISINFECTANTS.

Previous attempts to estimate the disinfecting value of light under varying conditions have been based on the time required for complete sterilization of a given quantity of liquid. Such a measure, however, would be of no value for designing a disinfecting apparatus; for in such an apparatus the water would necessarily be moving, and could not be kept under constant conditions until sterilized. Moreover, anyone familiar with the typical curve of disinfection (see Fig. 1) must recognize that the intersection of its flattest part with the horizontal axis must be difficult to determine with any accuracy whatever, and that any method of standardization based on such a determination must be unsatisfactory, even if there is any simple relation between this time and the bactericidal efficiency of the disinfectant. Cernovodeanu and Henri⁶ (1910) attempted to use this procedure, with the following results:

TABLE 1.

DISTANCE	TIME NECESSARY FOR STERILIZATION	
	110 v. Lamp	220 v. Lamp
	Seconds	Seconds
60	300	30
40	180	15
20	20	4
10	4	1

¹ *Compt. rend. Acad. d. Sci.*, 1910, 150, p. 932; *ibid.*, 151, p. 677.

² *Ibid.*, p. 1076.

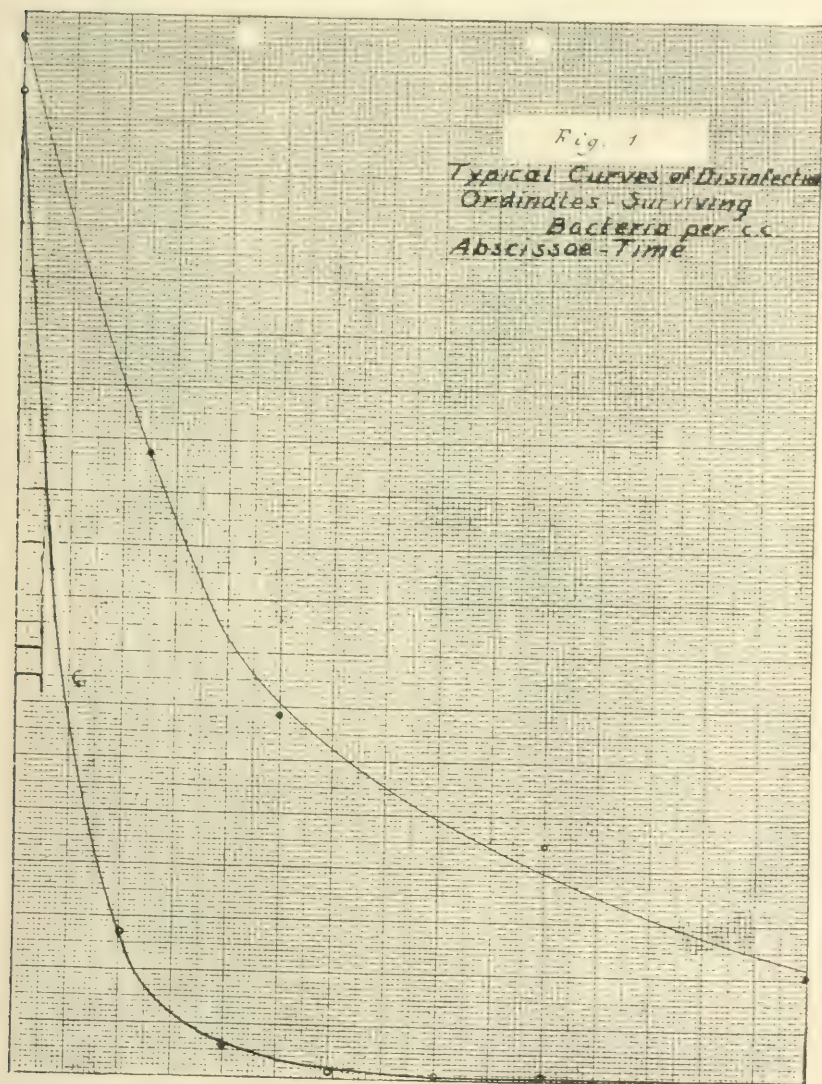
³ *Ibid.*, 151, p. 548; *ibid.*, p. 770.

⁴ *Ibid.*, 150, p. 52; *ibid.*, p. 549.

⁵ *Sterilization von Wasser mittels ultravioletter Strahlen*, Berlin, 1911.

⁶ *Loc. cit.*

They concluded that there is no simple relation between time of sterilization and distance, and pointed out that the time varied more rapidly than the square of the distances.



More applicable to the practical problem would be a measure of the instantaneous effect—the rate of disinfection. And such a method has been proposed for chemical disinfectants as a result of

studies inspired by the observations that disinfection is always an orderly process with respect to time, and that the curve of disinfection is always of the same general type (see Fig. 1). In 1907 and 1908, Madsden and Nyman,¹ and Chick,² working simultaneously but independently, demonstrated that, in the disinfection of anthrax spores by chemicals, the logarithms of the numbers of living spores varied inversely as the time. Both concluded that, in the case of anthrax spores, the disinfection proceeds in the same manner as a monomolecular chemical reaction—in other words, that the rate of disinfection varies directly as the number of surviving spores. This theory has been developed and elaborated by Phelps³ (1911) and supported by additional experimental evidence by Chick⁴ (1910).

The mathematical form of this law is $\frac{dN}{dt} = kN$ where “ N ” is the number of living bacteria in a unit volume. Integrating between times t_1 and t_2 , $\log \frac{N_2}{N_1} = k(t_2 - t_1)$, and the accordance of the law with the facts will be represented by the degree of constancy

of experimental determinations of $k = \frac{\log \frac{N_2}{N_1}}{t_2 - t_1}$.

The experiments of Madsden and Nyman⁵ (1907) on disinfection of anthrax spores with mercuric chloride, those of Chick⁶ (1908, 1910) on disinfection of anthrax spores with phenol, and a few of her experiments with *B. typhosus*, *B. coli*, and other organisms, show remarkable agreement in the values of the constant obtained. But in the great majority of Chick's experiments on vegetative cells, in her analyses of those of Kronig and Paul,⁷ and of Clark and Gage⁸ (see Chick, *Jour. Hyg.*, 1910, 10, pp. 239–80), and in 10 of my own experiments, a gradual decrease in the constant, often of very great magnitude, was noted with increasing values of “ t .” As examples, I quote below values obtained from one of Chick's experiments, and from one of my own. Chick has used common

¹ *Ztschr. f. Hyg.*, 1907, 57, p. 388.

² *Jour. Infect. Dis.*, 1911, 8, p. 1.

³ *Loc. cit.*

⁴ *Jour. Hyg.*, 1908, 8, p. 92; p. 655.

⁵ *Jour. Hyg.*, 1910, 10, p. 237.

⁶ *Loc. cit.*

⁷ *Ztschr. f. Hyg. u. Infektionskr.*, 1897, 25, p. 1.

⁸ *Rep. State Bd. Health, Massachusetts*, 1903, 34, p. 268.

TABLE 2.
DATA TAKEN FROM CHICK (1908), TABLE IX, P. 108.

TIME (MINUTES)	NUMBER BACTERIA IN ONE DROP OF DISINFECTION MIXTURE	VALUE OF $K = \frac{\log \frac{N_0}{N_t}}{t_2 - t_1}$		
		$t_1 = 0$	$t_1 = 5$	$t_1 = 10$
0.....	25,250	—	—	—
0.5.....	540	3.74	—	—
1.0.....	395	1.92	0.50	—
2.1.....	97	1.15	0.47	0.45
3.1.....	50	0.87	0.40	0.37
4.1.....	24	0.74	0.38	0.36
5.2.....	10	0.65	0.37	0.35
7.0.....	2	0.57	0.36	0.36, etc.

or Briggs logarithms in all her work. I follow her in quoting her results, but in all my calculations, based on my own experiments, natural or Napierian logarithms are used.

TABLE 3.
DISINFECTION OF *B. COLI* WITH ULTRA-VIOLET LIGHT FROM MAGNETITE ARC AT 20 CM. DISTANCE.

TIME (SECONDS)	NUMBER BACTERIA PER C.C.	VALUE OF $K = \frac{\log \frac{N_0}{N_t}}{t_2 - t_1}$			
		$t_1 = 0$	$t_1 = 2$	$t_1 = 4$	$t_1 = 6$
0.....	185,000	—	—	—	—
2.....	27,100	0.99	—	—	—
4.....	5,950	0.86	0.76	—	—
6.....	1,020	0.87	0.82	0.88	—
8.....	990	0.65	0.55	0.45	0.015
10.....	880	0.54	0.43	0.32	0.037
15.....	420	0.41	0.32	0.24	0.099
20.....	211	0.34	0.27	0.21	0.11

It is significant that this change is always in one direction, and its regularity, when K values are plotted against time, suggests at once some controlling law. Chick recognized this and comments,¹ "The decrease in the value of K in the case of *B. paratyphosus* is a regular and orderly one. If values of K are plotted against numbers of surviving individuals, a continuous curve is obtained, showing that the value of K is altering in accordance with some law, and bears some relation to the number of surviving bacteria." In 1910 she made a similar comment,² and suggested that this deviation was connected in some way with variation among the bacteria.

¹ *Jour. Hyg.*, 1908, 8, p. 109.

² *Ibid.*, 1910, 10, p. 282.

with respect to degree of possession of the property that causes disinfection to run logarithmically.

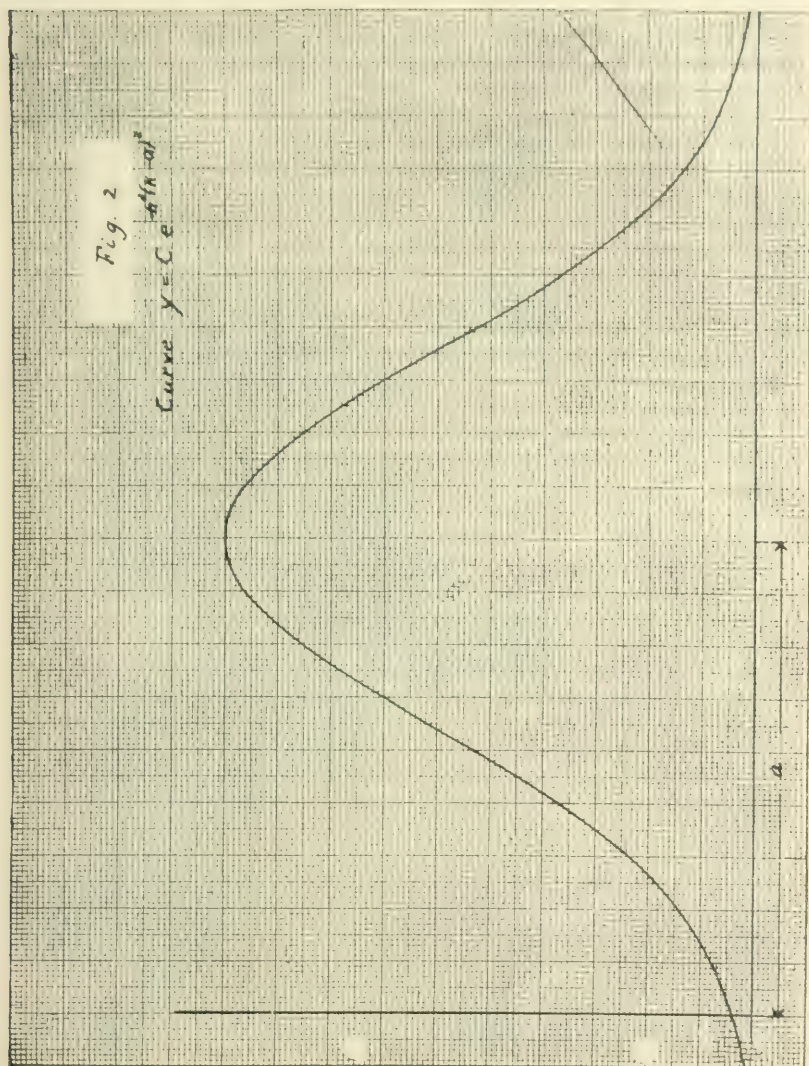
Following the line of this plausible suggestion, so much more in accordance with biometric experience than a theory based on equal possession of any natural property, I have developed the logarithmic theory of disinfection, assuming that any bacterial culture contains cells with varying K values, and that their distribution with respect to this property accords with biometric experience—namely, that there is a concentration of numbers about some mean or modal value, with smaller and smaller numbers departing extremely from this mode.

As a mathematical basis for this theory, two groups of curves were studied, both of which have been shown by biometricians to bear a strong general resemblance to the plotted curves of distribution of individuals with respect to biological characteristics. One, $y = Ce^{-h^2(k-a)^2}$ (see Fig. 2), is the “curve of error,” representing the distribution of the errors of experimental observation. The other family, $y = C \left(\frac{k}{a}\right)^n e^{-h^2\left(\frac{k}{a}\right)^2}$ (see Fig. 3), is a group of which the member where $n=2$ is the curve representing the distribution of errors of mean square, and is the basis of Maxwell’s kinetic theory of gases. In both cases, “ a ” is the value of “ k ” at the mode, and the area under any portion of the curve represents the number of organisms having “ K ” values between those corresponding to the limiting ordinates.

Without discussing the relative merits of these two sets of curves, or presenting the mathematical study that was made of both, it may be said that no analytical solution of either was found after several weeks’ work. And while it was shown that solutions of the former by trial and error, and of the latter by a graphical method, are possible, neither could be worked out without a great amount of labor. Only a very few values were obtained in this way, not enough to determine whether or not such values would show any greater constancy than the “ K ” values previously discussed. It

was demonstrated, however, that, in both cases, $\lim_{t \rightarrow 0} \frac{\log \frac{B}{b}}{t} = Dq$,

where " B " is the initial number in a unit volume, " b " is the number surviving in a unit volume after time " t ," " a " is the modal



value of " K " at the beginning of the disinfection, and " D " is a constant for the culture independent of the kind or concentration of the disinfectant. (For this demonstration, one assumption was

necessary, viz.: that the relative proportion having "K" values greater than the mode will be the same, whatever the absolute value of "a.") It is therefore possible to obtain the ratio of "a" values (*D* canceling out) for different disinfectants, or for different

conditions, by plotting $\frac{\log \frac{B}{b}}{t}$ against time, and extending the curves to intersect the axis $t=0$. As "a" represents the rate of action of the given disinfectant upon a specific organism, and not a composite value for a mixture of varying ones, it is reasonable that this ratio should represent a truer measure of the relative efficiency of the disinfectants, or of the same disinfectant under varying conditions, than the mean of the "K" values proposed by other investigators. And the constancy of "K" in the experiments on spores fits in with the theory through the reasonable explanation that there must be considerably less biological variation between the inert, resting cells, than between active, vegetative cells, carrying on a greater variety of processes and subjected to a greater number of selective influences.

EXPERIMENTAL.

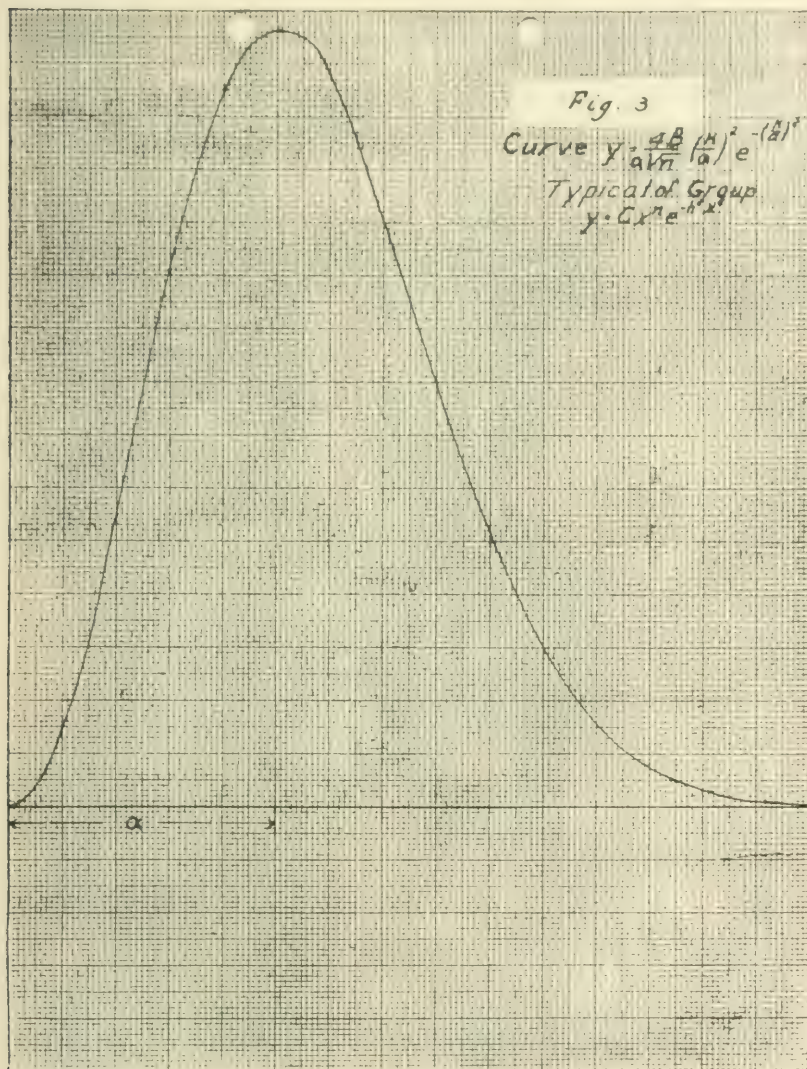
All the experiments made in this investigation were carried on with a laboratory stock culture of *B. coli*. A standard loopful was transferred every day to a tube of peptone, and a two-day old culture was used, except in one or two experiments. In general, the numbers in the two-day culture were reasonably constant, and between 100,000,000 and 200,000,000 per c.c., and a 1/1000 dilution in sterile water was used for the experiments.

Two sources of light were used. A magnetite arc without a globe, loaned by the Edison Electric Illuminating Co., of Boston, was connected with the 110 v., direct current circuit, and took about 6.6 amperes, on the average.

The other lamp was a Westinghouse-Cooper Hewitt quartz tube mercury lamp, made by the Westinghouse-Cooper Hewitt Co., of Paris, and loaned by the Cooper-Hewitt Co., of New York, for this investigation.

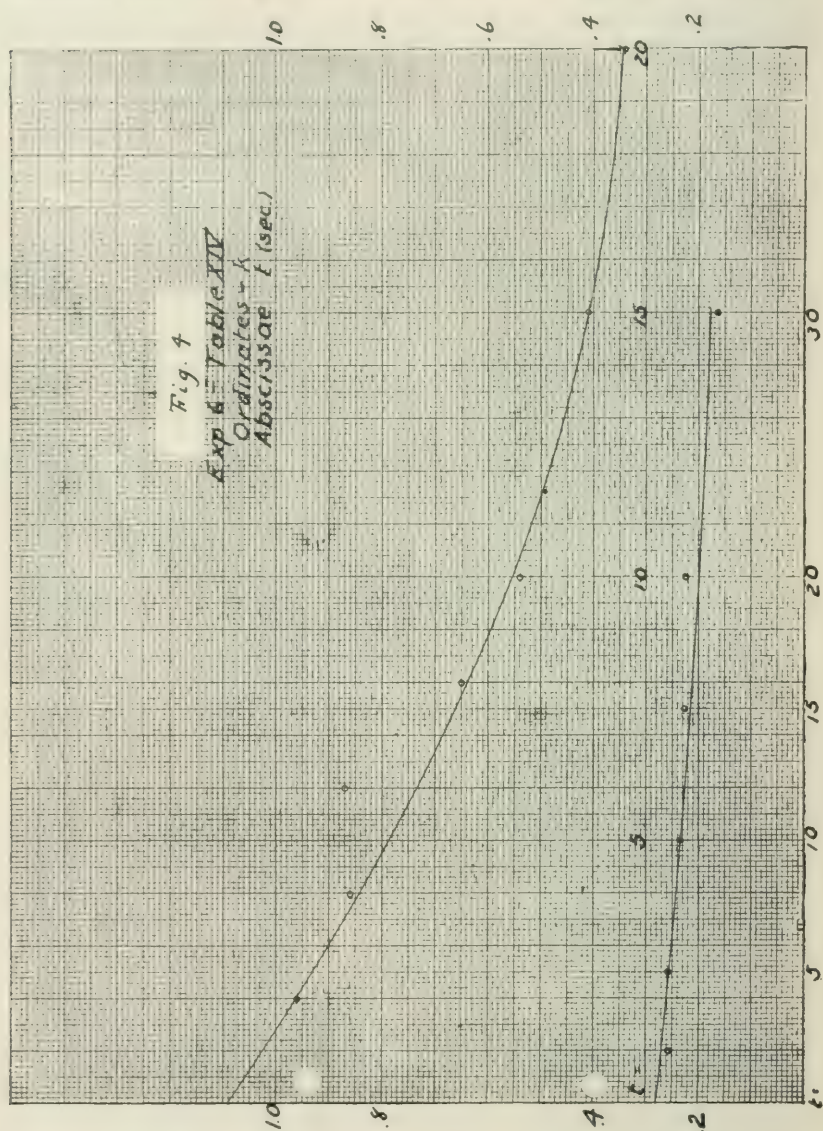
The lamp had a luminous tube about 12 cms. long and was connected with a 220-volt direct current circuit. The voltage was

cut down by interposing a resistance to 200 volts, and the lamp took, on the average, about 4.2 amperes.



The procedure finally adopted was as follows: About 1.2 c.c. of the 1/1000 dilution of the culture was pipetted into a quartz test tube, $\frac{3}{8}$ inches in diameter by about 3.5 inches long. The lamp

was lit, and the tube suspended in a ring in the proper position, and screened from the light with a slate screen. A test was started by



removing the slate screen quickly, and it was ended by throwing a switch and cutting off the lamp. Where it was impractical to

interpose the screen between the lamp and the tube, the tube was screened and held just above the ring. The test was then started by dropping the tube through the ring, and ended as before by cutting off the lamp. As soon as the lamp was cut off, the tube was removed, its mouth sterilized in a flame, and one c.c. of its contents transferred in a sterile pipette to the dilution water, or direct to a Petri dish if it was not to be diluted. Samples were plated with gelatin, incubated at 20° for 48 hours, and counted.

After several experiments, it was thought probable that growth in the diluted culture during the experiment might interfere with the work, and the plan was adopted of keeping it thereafter in a double walled vessel, packed with ice, so that the temperature was maintained at 8-10° C.

1. *On the effect of varying distance.*—Seven experiments were performed to study the effect of varying the distance in air from the

light. Of these, three gave enough values of $\frac{\log \frac{B}{b}}{t}$, showing the usual orderly decrease to allow the construction of reasonably good curves. As an example of these curves, Fig. 4 shows those corresponding to Experiment 6 below. The results of these three experiments are given below:

TABLE 4

Expt. 6, February 12, 1911. *B. coli*, two-day peptone. Samples rayed horizontally from magnetite arc, 110 v., 6.6 amp.

Time (Sec.)	Counts	Dil.	Mean No. per c.c.	$\frac{\log \frac{B}{b}}{t}$
Distance 20 cm.				
0	175, 230, 140	3	185,000	
2	238, 193, 202, 300	2	27,100	.06
4	61, 61, 53, 63	2	5,950	.86
6	135, 84, 98	1	1,020	.87
8	75, 98, 127, 102	1	990	.95
10	77, 90, 88, 91	1	880	.94
15	307, 540	0	420	.44
20	280, 158	0	210	.34
Distance 40 cm.				
0	168, 220, 251, 74	3	180,500	
2	104, 115, 122, 90	3	108,000	.155
5	590, 487, 430, 405	2	49,500	.289
10	108, 184, 110, 108	2	17,300	.284
15	558, 524, 832, 810	1	6,810	.228
20	202, 205, 167, 180	1	2,035	.224
30	130, 132, 138, 118	1	1,310	.164

TABLE 5.

Expt. 7, February 13, 1911. *B. coli*, four-day peptone. Samples rayed horizontally from magnetite arc, 110 v., 6.6 amp.

Time (Sec.)	Counts	Dil.	Mean No. per c.c.	$K = \frac{\log \frac{B}{b}}{t}$
Distance 20 cm.				
0	300, 296, 266, 280	3	288,000
2	32, 36, 44, 34	3	36,500	1.03
4	74, 77, 87, 98	2	8,400	.88
6	23, 22, 28, 20	2	2,325	.80
8	72, 90, 88	1	833	.73
10	125, 126, 111, 95	1	1,140	.56
15	200, 428	0	314	.46
20	267	0	267	.35
Distance 40 cm.				
0	352, 341, 385, 411	3	372,000	.25
2	231, 242, 225, 200	3	224,500	.25
5	1000, 1060, 968, 952	2	101,000	.26
10	324, 325, 324	2	32,400	.24
15	1528, 1570, 1495, 1235	1	14,570	.22
20	450, 878, 750, 810	1	7,240	.20
30	290, 178, 171, 172	1	2,030	.17

TABLE 6.

Expt. 11, March 9, 1911. *B. coli*, two-day peptone. Samples rayed horizontally from mercury arc, 200 v., 4.2 amp.

Time (Sec.)	Counts	Dil.	Mean No. per c.c.	$K = \frac{\log \frac{B}{b}}{t}$
Distance 20 cm.				
0	322, 270, 276	3	289,300
2	18, 22	2	2,000	2.50
4	118, 172	1	1,450	1.33
8	377, 370, 340	0	362	.81
Distance 40 cm.				
0 taken same as above	289,300
2	45, 57, 40	3	50,300	.87
5	156, 146	2	15,100	.59
10	615, 401	1	5,380	.40

Plotting $\frac{\log \frac{B}{b}}{t}$ against time, and extending the curves to meet the axis $t=0$, taking the intercept equal Da , we have, calling distance " L ":

TABLE 7.

Experiment	L_1	Da_1	L_2	Da_2	$\left(\frac{L_1}{L_2}\right)^4$	$\frac{a_1}{a_2}$
	cm.		cm.			
6	20	1.08	40	.28	4	3.9
7	20	1.18	40	.29	4	4.1
11	20	4.70	40	1.17	4	4.0

In two other experiments it was possible to plot curves only by discarding one observation in each, which deviated extremely from the regularity indicated by the other observed values. In these cases the results were as follows:

TABLE 8.

Experiment	L_1	Dd_1	L_2	Dd_2	L_3	Dd_3	$(\frac{L_1}{L_2})^2$	$\frac{d_1}{d_2}$	$(\frac{L_1}{L_3})^2$	$\frac{d_1}{d_3}$	$(\frac{L_2}{L_3})^2$	$\frac{d_2}{d_3}$
8.....	cm. 40	.42	80	.10	80	.055	4	4.2	16	8.3	4	3.5
9.....	20	.455	40	.195			4	2.3				

In the other two experiments, no plots of the results were possible.

2. *Relative efficiency of different sources of light.*—Two experiments were tried comparing the efficiency of the magnetite arc with that of the mercury arc. In neither case was it possible to construct " $K-t$ " plots for both, and no quantitative relation was established. The experiment below, however, indicates that the mercury arc is many times as effective, although using only 1.2 times as much power:

TABLE 9.

Expt. 13, February 19, 1911. *B. coli*, two-day peptone. Samples rayed at 40 cm. horizontally from source of light.

Time (Sec.)	Counts	Dil.	Mean No. per c.c.	$K = \frac{\log \frac{B}{b}}{t}$
Magnetite arc				
0.....	277, 273, 314, 288	3	288,500	...
2.....	231, 202, 221	3	218,000	.14
5.....	131, 124	3	127,500	.16
20.....	125, 129, 132, 103	2	12,200	.16
Mercury arc 4.4 amp., 198 v.				
0.....	379, 400, 347, 380	3	376,500	...
2.....	230, 256	2	24,750	1.36
5.....	6, 12	2	900	1.21

3. *Absorption in water.*—Three experiments were made on the effect of interposing between the lamp and the sample various thicknesses of distilled water. A cylindrical galvanized iron tank, about 6 cm. in diameter and one meter long, and open at the top, was made, and a quartz plate about 1.1 mm. thick was set in one

end in a water-tight rubber joint, fastened between brass plates with four binding screws. The plates had a window about 2 mm. square cut out. Experiments were made by placing about 1.2 c.c. of the diluted culture in a quartz test-tube, and dropping the tube through a ring, so placed that the lower end of the tube passed through the open top of the tank into the water. The ring was at such a height that the tube was supported so as to bring the sample opposite the center of the quartz window. With this apparatus tubes could be exposed through any thickness of water up to one meter.

TABLE 10.

Expt. 20, April 1, 1911. *B. coli*, two-day peptone. Samples rayed horizontally opposite mercury arc lamp, 4.1 amp., 200 v.

Time (Sec.)	Counts	Dil.	Mean No. per c.c.	$K = \frac{\log \frac{B}{b}}{t}$
Distance 40 cm. in air.				
0	210, 225, 226, 262	3	231,000	...
2	114, 116	3	115,000	.35
4	55, 55	3	55,000	.36
6	225, 245	2	23,500	.38
Distance 22.5 cm., 2.5 in air and 20 cm. in distilled water.				
0 taken same as above			231,000	...
2	155, 171	3	103,000	...
5	129, 126	3	127,000	.17
10	95, 153	2		.12
	20, 21	3	18,700	.25
20	18, 10	2	1,400	.25
Distance 32.5 cm., 2.5 cm. in air and 30 cm. distilled water.				
0 taken same as above			231,000	
5	136, 157	3	146,500	.092
10	107, 141	3	127,000	.060
20	280, 302	2	29,600	.103
Distance 42.5 cm., 2.5 cm. in air and 40 cm. in distilled water.				
0 taken same as above			231,000	
5	212, 207	3	209,500	.020
10	164, 144	3	154,000	.041
20	98, 85	3	91,500	.046
40	27, 45	3	36,000	.047

No satisfactory plots were possible, but as a rough measure of the order of magnitude of the relation, the means of these " K " values may be compared, assuming the values at the same distances in air to vary inversely as the square of the distance:

TABLE 11

Distance (cm.)	Length Water Column (cm.)	Mean Value K^{-1}	Calculated K^{-1} at Same Distance in Air	Absorption (Percentage)
10	2	36		
22.5	22	25	1.11	82.5
32.5	32	28.5	1.77	84.5
42.5	42	25.9	2.22	88.0

The quantitative results cannot be supported in accordance with the method of treatment previously adopted; but they serve to indicate that absorption is greatest in the first layers, increasing slowly with increased thickness of water.

One experiment was tried on the absorption in Boston tap water, which had a color of about 0.4.

TABLE 12.

Expt. 21, April 2, 1911, *B. coli*, two-day peptone. Samples rayed at 22.5 cm. horizontal (2.5 cm. in air and 20 cm. tap water, color 0.4) from mercury arc lamp, 4.2 amp., 200 v.

Time	Counts	Dilution	Mean No. per c.c.
0	176, 192, 205, 182	8	170,000
	177, 161, 120, 182		
2	178, 104	8	184,000
5	208, 170	8	189,000
10	168, 165	8	165,000
20	164, 176	8	170,000

The absorption appears to have been complete, which is in accordance with the work of Courmont and Nogier,¹ (1909) on the impermeability to ultra-violet light of water containing colloidal matter.

CONCLUSIONS.

The method proposed for measuring the bactericidal effect of a disinfectant is a reasonable one, and slight support is given to it by the fact that, for those cases in which satisfactory plots were drawn, the calculated ratios of the rates of disinfection for the modal bacteria were closely inversely as the square of the distance, or as the intensity of light. It should be noted, however, that of the 10 experiments in which it was possible to plot " $K-t$ " curves, five were preliminary experiments, and five were on the effect of varying distances in air. In the absorption experiments, in those on different sources of light, and in a few others, an irregular

¹ *Compt. rend. Acad. d. Sci.*, 1909, 120, p. 302.

variation in calculated " K " values, or lack of sufficient determinations, make it impossible to make satisfactory plots. Part of this may have been due to failure to make proper dilutions, through inability to predict the effect of these new conditions, and consequent loss of plates. But in some instances an ample number of points was determined, though varying so irregularly as to preclude plotting; and in a few cases an increase in the calculated

values of $K = \frac{\log \frac{B}{b}}{t}$, instead of a decrease, was noted. No satisfactory explanation of these irregularities has suggested itself. Nevertheless, it is believed that the method is reasonable, and sufficiently novel and interesting to justify its presentation, not as a proved fact, but in the hope that it may lead to further study and experiment.

Granting the reasonableness of the method, some evidence is presented that the disinfecting power of ultra-violet light varies as the intensity of incident energy, or inversely as the square of the distance. And this has some further support in the fact that the absorption in water increases very slowly with increased distance, suggesting analogy to the law that the absorption of actinic energy varies directly as its intensity. If this is true, no analytical solution of the effect on water moving toward a light can be made, for no coefficient of absorption can be determined, except for monochromatic light. It would follow, then, that no rational design of an apparatus for disinfecting water can be made, but that the machine must be constructed in accordance with certain general principles, and modified to attain maximum efficiency by experiment.

The mercury arc lamp is more effective than the magnetite arc. There is reason to believe that its efficiency may be increased by varying the voltage, and the point of maximum efficiency would have to be determined experimentally.

Disinfection by ultra-violet light would be applicable to surface waters, containing vegetable coloring matter only if the color were first removed by coagulation or filtration.

A STUDY OF THE ACTION OF ANTISTREPTOCOCCUS SERUM IN STREPTOCOCCUS INFECTIONS IN MAN.*

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(From the Memorial Institute for Infectious Diseases, Chicago.)

In our earlier study of antistreptococcus serum[†] we found that the blood serum of persons with streptococcus infections, although it might be deficient in streptococcus opsonin, was still able to activate antistreptococcus horse serum. From this we inferred that antistreptococcus serum would probably have immunizing and curative action against living streptococci in man. The present report has to do with some studies of phenomena arising after injections of antistreptococcus serum in patients infected with streptococci. We have not undertaken the treatment of a large number of cases so as to arrive at results by statistics. Our purpose has been to determine whether in patients with streptococcus infections, injections of antistreptococcus serum were followed by an appreciable increase of antistreptococcus bodies in the blood, and if so, whether coincident clinical improvement was apparent. Since streptococcus infections in man run such variable courses and so large a proportion terminate in spontaneous cure, it has been difficult to arrive at definite conclusions as to the real therapeutic value of antistreptococcus serums. Some clinical observers have been enthusiastic as to the results obtained while others have only met with disappointment. We have hoped that the present study might aid in clearing up some of this uncertainty. If the administration of the serum should be followed simultaneously by an increase in antistreptococcus bodies in the blood and by an amelioration of the symptoms of disease, it would be natural to infer that both were brought about by the action of the serum. It must, however, be remembered that in the treated cases we have a combination of a passive immunity produced by the injected antibodies and of an active immunity due to the action of the infecting bacteria.

* Received for publication April 10, 1912.

† *Jour. Infect. Dis.*, 1911, 9, p. 130.

In our former studies in guinea-pigs we found that the increase of opsonin in the blood after injections of antistreptococcus serum was a fairly accurate guide to the protection afforded. In the cases here reported we have studied the variations in streptococcus opsonin and also the phagocytic activity of the leukocytes subsequent to injections of antistreptococcus serum. Erysipelas and scarlet fever were selected as being the diseases of which a sufficient number of cases were available so that very severe examples might be chosen. A modified Wright's technic for determining the opsonin was followed, using a streptococcus culture which had lost much of its virulence under cultivation. The antistreptococcus serum employed was of two lots of polyvalent serum, kindly furnished for the purpose by Dr. E. M. Houghton, director of the Biological and Research Department of Parke, Davis & Co., to whom we acknowledge our obligation. Before using, the activity of the serums was determined in two ways. One part of the serum was added to nine parts of fresh, normal human serum. Such mixtures when diluted from 48 to 96 times with salt solution opsonized streptococci more than did similar dilutions of corresponding mixtures of normal serums. Also, 1 c.c. of the serums protected guinea-pigs against a fatal dose of living streptococci given 18 hours subsequently.

Early in our study we were fortunate in an opportunity of observing the effects of injections of antistreptococcus serum in a woman in whom it was desirable to produce a streptococcus immunity before the performance of an operation for removal of a hypophyseal tumor by the nasal route, since erysipelas seems very apt to follow such operations. The case was in the charge of Dr. Allen B. Kanavel, to whom we are indebted for the privilege of making these observations. Fifty c.c. of antistreptococcus serum were injected the day before and two days subsequent to the operation. Chart 1¹ shows the course taken by the streptococcus index and by the activity of the leukocytes toward streptococci.

¹ In connection with all the charts in this article "opsonic index" is used to indicate the streptococcus opsonic power of the patient's serum compared with that of normal serum; "cytophagic index" is used to indicate the phagocytic power of the patient's leukocytes with respect to streptococci compared to that of normal leukocytes under the influence of normal serum. The temperature curve is constructed from the highest temperature for each day.

Similar to what is observed in guinea pigs, there was a rise in the opsonic index and in the activity of the leukocytes. Unfortunately, there developed a pneumococcus meningitis which was fatal. It is likely that this may have modified the curves to some degree after the second day. This observation shows that in man, as in guinea-pigs, a marked rise in opsonin follows the injections of anti-streptococcus serum.

Alterations in the streptococcus opsonin and in the phagocytic activity of the leukocytes subsequent to injections of antistreptococcus serum were studied in six cases of erysipelas, two cases of septic scarlet fever and two cases of chronic otitis following scarlet fever. The former eight cases were of a severe type and selected as being likely to run unfavorable courses.

The course taken by the opsonic index, by the phagocytic activity of the leukocytes, and the temperature in the cases of erysipelas which were given the serum and in our uninjected cases is shown in the appended charts (Charts 2 to 7) to which brief clinical notes are added. All of the six cases terminated in recovery, and visible improvement usually began about 24 hours after the serum was given. The improvement was shown by falling temperature, recession of the local swelling, subsidence of delirium, and betterment in the general condition. In short, the impression upon the observers was that recovery was more rapid than could be expected in the usual course of the disease in similar cases. That the rapidly favorable outcome was

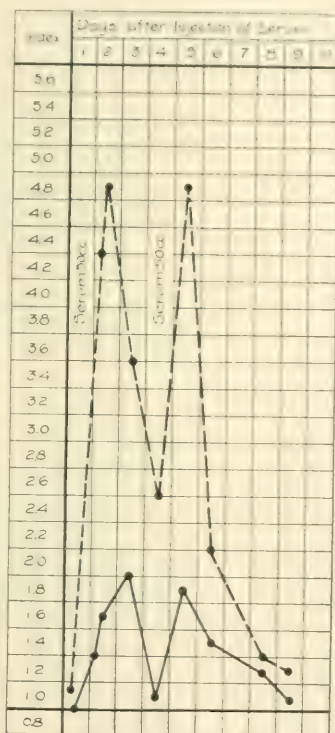


CHART 1.—Showing the opsonic index for streptococci and the increase in the phagocytic power of the leukocytes after the intramuscular injection of antistreptococcus serum in the case of Dr. Kanavel's patient with tumor of the hypophysis.

Solid line = opsonic index.

Broken line = phagocytic power of the leukocytes of the patient as compared with the leukocytes from a normal person (cytophagic index).

not accidental was further indicated by the concurrent increase of streptococcus opsonin above that observed in untreated cases. This increase of opsonin was noted at the time when gradual improvement began (Charts 3 and 4) or was sometimes delayed a short

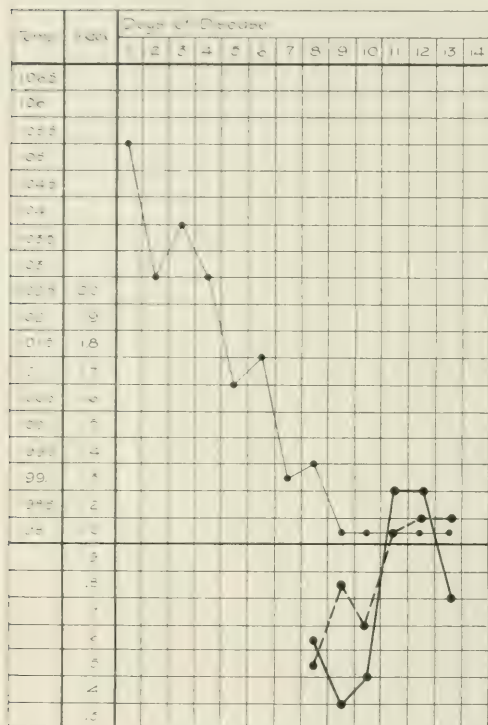


CHART 2.—Severe erysipelas, no serum, recovery. Case 1.
Heavy solid line = opsonic index.

Heavy broken line = phagocytic power of patient's leukocytes as compared with that of normal leukocytes (cytaphagic index).

Fine solid line = temperature.

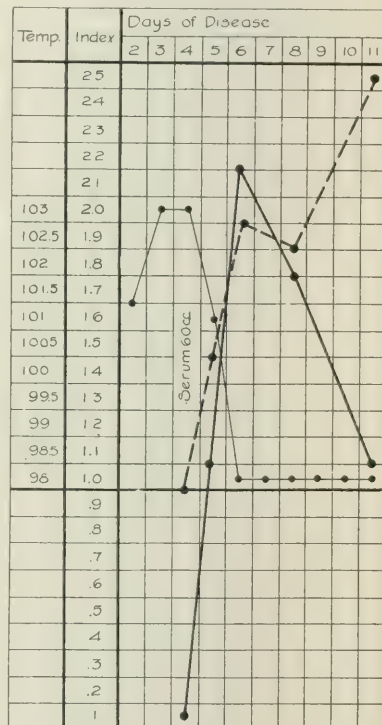


CHART 3.—Severe erysipelas, injection of serum on the fourth day, recovery. Case 2.
Heavy solid line = opsonic index.

Heavy broken line = phagocytic power of patient's leukocytes as compared with that of normal leukocytes (cytaphagic index).

Fine solid line = temperature.

time or rose very slowly at first, as if the index rose only when the infection was effectually checked (Charts 5 and 6).

In the case represented by Chart 7, the indexes were not taken before the serum was given. It is likely that the alteration on the day following the injection was brought about by the serum, but the subsequent course corresponds to that noted in untreated cases.

The degree of passive immunity was not sufficient to check the infection and an active immunity was finally developed.

The serum was administered to two cases of septic scarlet fever. In each there was a leukocytosis and in each the leukocytes

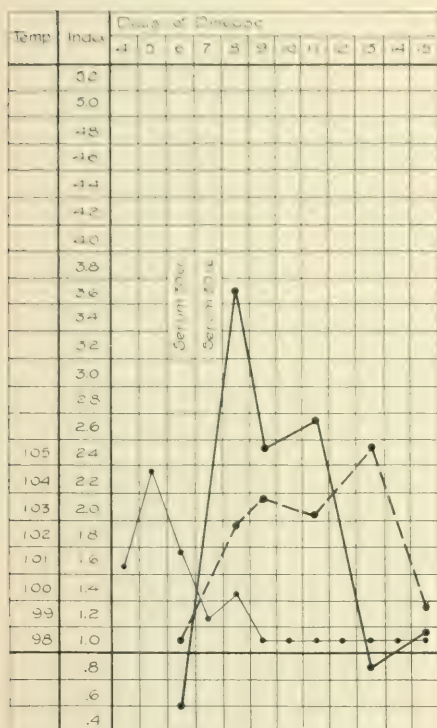


CHART 4.—Severe erysipelas, injection of serum on sixth and seventh days, recovery. Case 3.

Heavy solid line=opsonic index.

Heavy broken line=phagocytic power of patient's leukocytes as compared with that of normal leukocytes (cytophagic index).

Fine solid line=temperature.

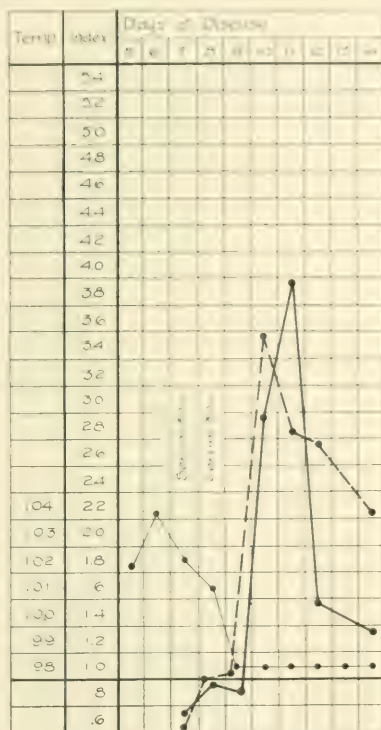


CHART 5.—Severe erysipelas, injection of serum on the seventh and eighth days, recovery. Case 4.

Heavy solid line=opsonic index.

Heavy broken line=phagocytic power of patient's leukocytes as compared with that of normal leukocytes (cytophagic index).

Fine solid line=temperature.

showed increased phagocytic activity before the injection. In each, the opsonic index was subnormal, but rose after the serum was injected, and with this rise the temperature fell and the general condition improved. Both cases were desperately sick with a very bad outlook before the serum was given, and both made as rapid and complete a recovery as occurs usually in untreated cases of

moderate severity. Subjoined are charts of the observations in these cases with brief clinical notes, and also for comparison a similar chart from an uninjected case (Charts 8, 9, 10).

In two cases of chronic suppurative otitis media following scarlet fever in which cultures from deep in the ear yielded streptococci in

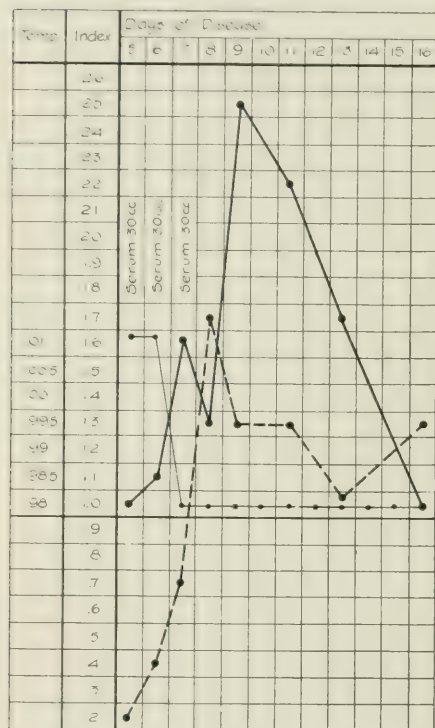


CHART 6.—Severe erysipelas, injection of serum on the fifth, sixth, and seventh days, recovery. Case 5.

Heavy solid line=opsonic index.

Heavy broken line=phagocytic power of patient's leukocytes as compared with that of normal leukocytes (cytophagic index).

Fine solid line=temperature.

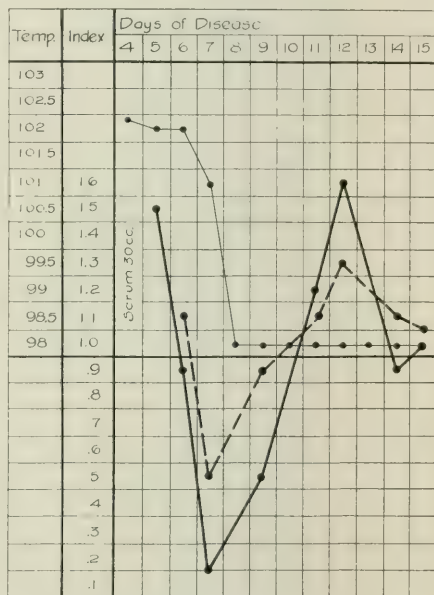


CHART 7.—Severe erysipelas, injection of serum on the fourth day, recovery. Case 6.

Heavy solid line=opsonic index.

Heavy broken line=phagocytic power of patient's leukocytes as compared with that of normal leukocytes (cytophagic index).

Fine solid line=temperature.

almost pure growth, a single injection of 60 c.c. was given. Both children had received diphtheria antitoxin several weeks before, and they both developed a severe serum reaction a few hours after the injections. In each case the opsonic and cytophagic indexes rose very considerably and remained elevated several days. The effect upon the aural discharge was not pronounced.

We have not found that the injection of serum produces an increase in leukocytes. However, a leukocytosis already present falls after the administration of the serum as symptoms abate (Chart 9).

The increased activity of the leukocytes following the serum

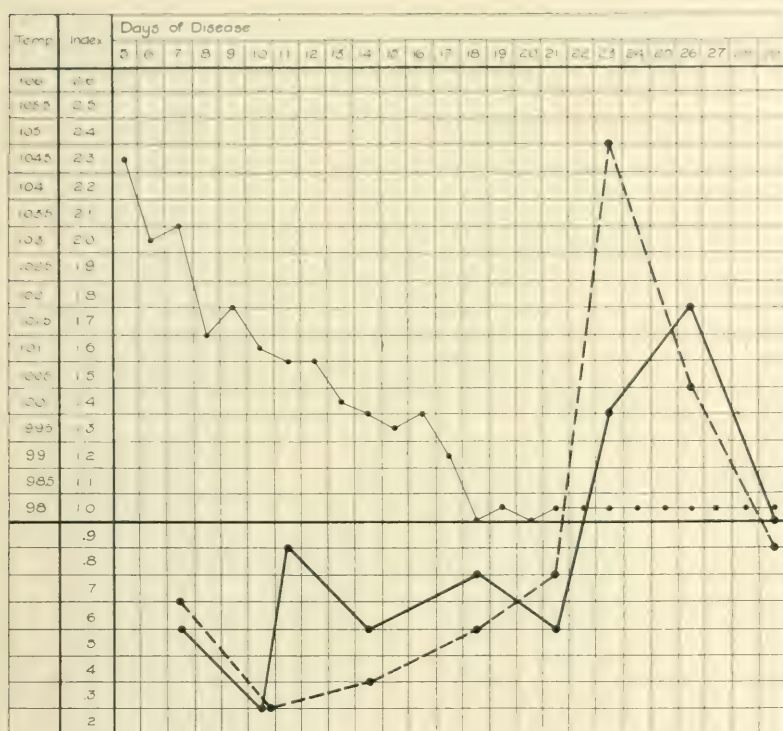


CHART 8.—Septic scarlet fever, no serum, recovery. Case 7.

Heavy solid line=opsonic index.

Heavy broken line=phagocytic power of patient's leukocytes as compared with that of normal leukocytes (cytophagic index).

Fine solid line=temperature.

injections does not appear to be specific, as we had thought probable in our former study in guinea-pigs.

Most of the injections have been made into the muscles to facilitate rapid absorption. No untoward effects were noted after such injections. In very urgent cases the intravenous injection should be considered as likely to yield the quickest results.

An experiment was made (Tunncliffe) to determine the concentration of streptococcus opsonins in the blood of rabbits following the intravenous, intramuscular, and subcutaneous injections of

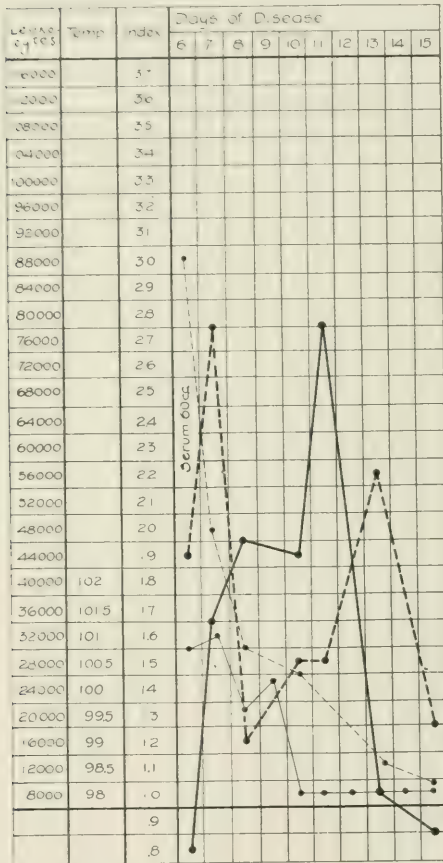


CHART 9.—Septic scarlet fever, injection of serum on the sixth day, recovery. Case 8.

Heavy solid line = opsonic index.

Heavy broken line = phagocytic power of patient's leukocytes as compared with that of normal leukocytes (cytophagic index).

Fine solid line = temperature.

Fine broken line = leukocytes.

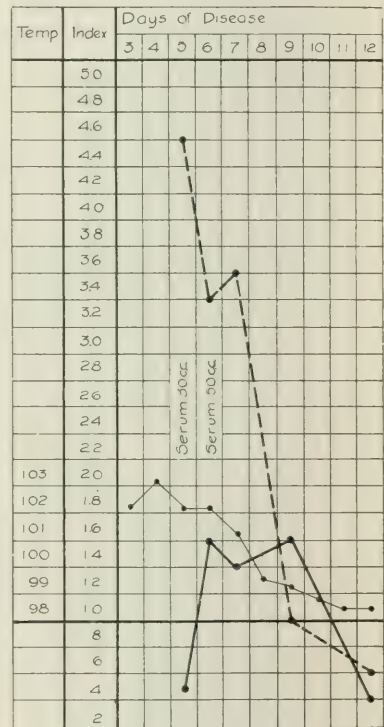


CHART 10.—Streptococcic scarlet fever, injection of serum on fifth and sixth days, recovery. Case 9.

Heavy solid line = opsonic index.

Heavy broken line = phagocytic power of patient's leukocytes as compared with that of normal leukocytes (cytophagic index).

Fine solid line = temperature.

4 c.c. of antistreptococcus serum. The opsonic index rose in 10 minutes following the intravenous injection, in two hours after the intramuscular injection, and in 24 hours after the subcutaneous

injection. The highest index produced by the intravenous injection was 3.9; by the intramuscular, 2.5; and by the subcutaneous, 1.9. The rise persisted in each case for 24 hours.

The dose of serum may be said to be entirely arbitrary. Our experience corresponds with that of other observers in pointing toward rather large doses as required to produce results. This is also suggested by the relatively large quantities of serum necessary to bring about immunity in experimental animals. As a guide to the amount of serum required by the individual case, the opsonic index appears of value. When a rise of the opsonic index after the injection of serum is promptly followed by a fall below normal, even though associated with some amelioration in the clinical symptoms, it would seem best to give more serum at once. In similar manner a persistent leukocytosis may be found useful as pointing to the need of further administration of serum.

In our former paper we showed that the commercial antistreptococcus serums vary considerably in the amount of antibodies they contain. It is therefore to be expected that they will vary in their effects and that one will accomplish certain results in much smaller doses than another. We also called attention to the fact that the antibodies may be secured in higher concentration by a process of precipitation similar to that employed in the case of antidiphtheric serum. It would be desirable to know whether such concentrated serum would possess higher therapeutic value which might make it possible to administer larger amounts of specific antibodies in smaller bulk.

There is still lacking any definite standardization of antistreptococcus serum, upon which the dosage must be finally dependent. Such standardization would enable the producer to select the horses which yield antibodies in the largest amount.

CASE 1 (Chart 2).—Man, 38 years; on the day of admission erysipelas started in a wound on the back of the neck produced by the surgical removal of a lipoma. The process extended until it involved the entire face, ears, and most of the scalp, reaching a standstill in about a week, followed by uninterrupted recovery.

CASE 2 (Chart 3).—Boy, 6 years; admitted on the second day of illness with typical erysipelas of the entire face, there being extensive blebs on the forehead and one eye entirely closed from swelling of the lids. The border of the area was sharply defined. On the two following days the swelling extended and the general symptoms became more severe. As the temperature fell rapid improvement took place in both

local and general condition and he was discharged entirely well 12 days after the onset of the attack.

CASE 3 (Chart 4).—Man, 39 years; attack began four days before admission with swelling on the right side of the face. When admitted there was a well-developed erysipelas of the whole face, one eye being entirely closed; patient nervous, muscles twitching; rapid, general and local improvement came with the fall of the temperature, convalescence being prompt and uncomplicated. Before the first injection the leukocytes were 9,600; before the second injection, 10,200, and 24 hours after, 5,600.

CASE 4 (Chart 5).—Man, 37 years; five days before admission there appeared a swelling of the nose which later extended to one side of the face and then to the other. On admission most of the face was involved in a typical erysipelas with sharply defined borders. On the two following days the swelling extended to the ears and scalp accompanied with much pain and general nervous symptoms; when the serum was injected the patient was actively delirious. The first injection was given at 11:30 P.M. and on the following morning there was no delirium, the temperature had fallen, and general improvement had set in. Recovery was rapid and uninterrupted. Leukocytes before the first injection were 8,400 and before the second injection, 7,100.

CASE 5 (Chart 6).—Man, 38 years; five days before admission the patient pricked a small papule on the lobe of the right ear; three days later the ear was swollen and tender and the swelling extended so that on admission the upper part of the face, including the forehead and both ears, was involved, the eyes being nearly closed. The erysipelas was typical, with sharply defined borders. As the temperature fell the local and general conditions rapidly improved, recovery being prompt and uncomplicated.

CASE 6 (Chart 7).—Man, 45 years; four days before admission chills followed by fever; coincidentally swelling began beside the nose and extended over the entire face and ears with enlargement of the glands behind the right ear. The patient appeared to be in a septic condition. The day after the injection the fever began to fall and both local and general symptoms to improve. The improvement however was slow and recovery took place in about two weeks. In this case the curve of the opsonic index corresponds to that observed in untreated cases, remaining below normal for some days after improvement had commenced and rising above normal only for a short time when convalescence was well established. It would seem that in this case not enough serum was injected to alter the course of the disease appreciably.

CASE 7 (Chart 8).—Man, 17 years; five days before admission he became suddenly sick with intense headache, vomiting, and fever. The next day there was a red rash. On admission he was very sick and delirious, conjunctivae injected, cervical glands enlarged, papillae of the tongue prominent, and diffuse typical rash. There were no diphtheria bacilli in the cultures from the throat. On the tenth day of the disease a purulent discharge from one ear appeared. Improvement came gradually and the recovery was complete.

CASE 8 (Chart 9).—Man, 22 years; severe angina five days before admission, and at about the same time there was noticed an eruption on the skin. When admitted there was a typical scarlatinal rash over the whole body; papillae of the tongue enlarged; mouth and lips very sore; teeth covered with sores; conjunctivae injected; muco-purulent discharge from the inner canthus. The glands and periglandular tissues on the right side of the neck enormously swollen from the clavicle to the lower jaw. Pulse 120. Marked nervous symptoms, the patient being irrational and actively delirious. No diphtheria bacilli in the throat. 60 c.c. antistreptococcus serum

injected intramuscularly. On the following day the swelling had extended up on the face, over the angle of the jaw; the patient apathetic and unconscious. Incision into the swollen tissues of the neck yielded only serum. On the next day, two days after the injection of the serum, the patient was resting quietly, was rational, and took food. There was a slight purulent discharge from the incision in the neck. From now on there was rapid and uninterrupted improvement, the wound in the neck closing in 10 days. The discharge from this wound was profuse, the cultures of the pus giving hemolyzing streptococci in pure growths.

CASE 9 (Chart 10).—Man, 18 years; ill several days before admission but the rash had been out only for 24 hours; when admitted there was a general typical scarlet fever eruption and severe angina with a false membrane on one tonsil. No diphtheria bacilli were found on repeated examinations. He was very toxic, irrational, and actively delirious with involuntary evacuations. Ten hours after the first injection he was much better, sleeping quietly, and seemingly improved in every way. From this time improvement progressed rapidly. On the 10th day there appeared acute swelling of the cervical glands which disappeared in a few days without suppuration. Leukocytes before the first injection were 15,600 and before the second injection, 20,600.

ON THE TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING. A STUDY UPON SERUM HEMOLYSINS IN GOATS.*†

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OUTLINE.

- I. INTRODUCTION: THE INHERITANCE PROBLEM; ITS RELATION TO DISEASE AND IMMUNITY; FUNDAMENTAL STUDIES; CONCLUSIONS.
- II. GENERAL REVIEW OF WORK UPON THE SUBJECT OF TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING.

A. ANTIBODIES OF WELL DIFFERENTIATED TYPES.

1. *Antitoxins induced by:*

- a) Plant toxalbumins: (1) Abrin; (2) Ricin; (3) Robin.
- b) Bacterial, extracellular toxins: (1) Tetanus; (2) Diphtheria; (3) Pyocyanus; (4) Symptomatic anthrax; (5) *Vibrio Nasik*.
- c) Animal venoms.
- d) Ferments.

2. *Agglutinins induced by:*

- a) Bacteria: (1) Typhoid (clinical observations and animal experimentation); (2) Tuberculosis (clinical observations); (3) Cholera; (4) *Proteus*.
- b) Blood. (1) Sheep corpuscles.

3. *Precipitins induced by:*

Sera.

4. *Cytolysins induced by:*

- a) Bacteria.
- b) Blood corpuscles.

5. *Opsonins induced by:*

Bacteria.

6. *Allergins induced by:*

Sera.

B. ANTIBODIES OF POORLY DIFFERENTIATED TYPES.

1. *Bacterial, intracellular toxins:*

- a) Organisms: (1) Anthrax; (2) Swine erysipelas; (3) Tuberculosis; (4) Dysentery.

2. *Spirilla:*

- a) *Sp. Obermeieri*.
- b) *Sp. pallida*.

3. *Protozoa:*

- b) Piroplasmosis (dog).
- b) Rabies.

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4. *Ultramicroscopic organisms:*

- a) Sheep-pox (ovine).
- b) Foot and mouth disease.
- c) Small-pox and vaccinia.

III. DISCUSSION OF RESULTS.

IV. REPORT UPON ADDITIONAL WORK CONCERNING TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING.

1. Introductory remarks: factors in passive transmission of antibodies; group of so-called intracellular bacterial toxins; immunity against intracellular toxins; complexity of this immunity process; cytolytic bodies as index to serum value; use of hemolysins in such studies.
2. Review of work reported upon transmission of hemolytic antibodies.
3. Choice of experimental animals and outline of technic.
4. Groups of experiments:
 - a) Active immunization of the adult before birth of the young, either during the period of gestation or before conception had taken place.
 - Series 1. Preliminary experiments.
 - Series 2. Special experiments.
 - b) Active immunization of the adult following immediately or shortly after the birth of the young.
 - Series 3. Animals normal at time of parturition.
 - Series 4. Animals partly immunized at time of parturition.
 - c) Active immunization of the newly born or older kid against foreign blood cells.
 - Series 5. Blood injections into kids.
 - d) Gastro-intestinal absorption of antibodies.
 - Series 6. Feeding of hemolytic serum to newly born kids.
5. General discussion of results.
6. Summary.

BIBLIOGRAPHY.

I. INTRODUCTION.

The inheritance, by the progeny, of newly acquired biological qualities from either or both parents has been a question of long standing. It has attracted the attention of numerous investigators, particularly physicians, since the beginning of scientific medicine and biology. Certain tendencies to disease were considered to be transmissible from parents to offspring (inherited). On the other hand, observations were recorded of cases where the mother, shortly after having recovered from a disease, gave birth to a child, and the child upon exposure to the same disease showed resistance against the infection. As an example of this character may be mentioned variola (Lereboullet, Buchner).¹ Burckhardt² made similar observations upon infants born from mothers

vaccinated (vaccinia) during late pregnancy. In small-pox an intrauterine infection of the fetus may occur, in which case not a true inherited, but an actively acquired, resistance results in the child; possibly the same thing occurs in vaccinia. The transient resistance which infants commonly show against many infections during the first weeks of life, while not fully understood, is probably for the most part of maternal origin.

As is evident, the problem has been beset with difficulties, and very little progress was made toward a solution until the newer discoveries in immunity became known. Immunity actively acquired against specific infectious agents or certain toxins offered itself as a means, readily applicable, by which an insight might be gained concerning the general laws of inheritance, and at the same time afford practical data on the subject of immunity. Following the discovery of the exciting causes of many of the infectious diseases, and the isolation of these specific organisms, animal experimentation was undertaken by several investigators. Chauveau,³ in 1880, found that by vaccinating with anthrax organisms the slightly susceptible Algerian sheep, during gestation, their offspring were born immune against this organism. These results were supported later by other workers using symptomatic anthrax (Arloing, Cornevin, and Thomas,⁴ Kitasato⁵).

A means had now been acquired by which fairly definite tests could be employed under conditions subject to control. More exact methods were introduced for actively immunizing animals against various antigens. The resulting degree of immunity became subject to standardization, thus establishing a firmer basis for studying the problem. Subsequently much important work appeared which threw more light upon the question.

The publication, in 1892, of the results of Ehrlich's⁶ basic studies marked a new epoch in the development of the subject. He carried out immunization experiments by feeding mice the toxalbumins, abrin, ricin, and robin. It was found that the young from immune mothers acquired an immunity, which he considered as passive, that is, a passage over of the specific antibodies to the fetus before birth, and then through the milk to the nursling after birth. He proved by placing normal young upon immune nurses, that the milk transmitted specific antibodies which were absorbed by the

nurslings, and caused their immunization. Also, he found that highly immunized males, when crossed with normal females, failed to endow the young with resistant powers against the specific toxin, and so concluded that the idioplasm of the sperm did not carry over immunity to offspring.

In opposition to Ehrlich's assertion that the immunized male alone was unable to transmit an acquired immunity to his descendants, several workers brought forward experiments which conflicted with his results. Among these workers may be mentioned Gley and Charrin,⁷ Tizzoni and Cattani,⁸ and Tizzoni and Centanni.⁹ Ehrlich and Hübener¹⁰ (1894) repeated similar experiments, using tetanus toxin against both guinea-pigs and mice, with results which sustained Ehrlich's first findings. In a critical review of the opposing work, they pointed out certain experimental conditions in each case which they considered invalidated the results. The investigations of Wernicke¹¹ (1895), Vaillard¹² (1896), and others (Remlinger,^{13, 65} Dieudonné,¹⁴ Bulloch,¹⁵ etc.) fully supported Ehrlich's findings. So at the present time the view is, for the most part, generally accepted that acquired immunity is not inherited by the offspring in the ontogenetic sense. In the mammalia apparently the germ plasm of neither parent plays an appreciable rôle in transmitting an acquired immunity to young.*

In discussing the question of the transmission of an acquired immunity from parents to the offspring, attention must be directed to the mother. The specific immunity of the offspring is a passive condition, and not a biological inherited condition of long standing. Therefore the subject may be transferred from the province of biological heredity (germ plasm) to the field of practical medicine and prophylaxis, and be considered from that standpoint.

II. GENERAL REVIEW OF WORK UPON THE SUBJECT OF TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING.

An extensive literature pertaining to the transmission of immunity from mother to offspring has developed in the past two decades. Since I have had occasion to make a rather exhausted

* It may be of interest to mention in this connection the work of Klemperer (*Arch. f. exper. Path. u. Pharmacol.*, 1893, 31, p. 356), who showed that the yolk of eggs from hens which had been actively immunized against tetanus showed antitoxin. Dzierzgowski (*Centralbl. f. allg. Path. u. path. Anat.*, Ref., 1901, 12, p. 715) confirmed these results with hens actively immunized against diphtheria. He states that chicks hatched from such eggs showed diphtheria antitoxin in their blood serum.

review of this literature, perhaps it may be of interest if a brief outline of the more important papers dealing with the subject be submitted. Special consideration will be given those papers which are important either from a historical standpoint, or those of fundamental value in the development of the subject. No attempt will be made to abstract even briefly the numerous articles, but instead a provisional classification of antibodies and antigens will be outlined, and under these headings the references will be catalogued. These papers, in general, concern the transmission of specific antibodies to the young by actively immunized animals. In some instances, simply the presence of antibodies in the milk of such actively immunized animals are reported. Purposely the work upon passive immunization of the mother animal is omitted, since in our studies attention is directed only to actively immunized mothers and their offspring.

A. ANTIBODIES OF WELL DIFFERENTIATED TYPES.

1. *Antitoxins*.—Animal experimentation has been carried out upon the following subgroups of toxins: a) Plant toxalbumins. As mentioned earlier, animals immunized against abrin, ricin, and robin transmitted the antibodies to their own young, or to normal sucklings (Ehrlich⁶).

b) Bacterial, extracellular toxins. (1) Tetanus. Actively immunized rats and rabbits (Tizzoni and Cattani¹⁶), guinea-pigs and mice (Ehrlich and Hübener¹⁰), and mare (Ransom¹⁷) transmitted an immunity to their offspring. It is of historical interest that Brieger and Ehrlich,¹⁸ in a study upon antitoxin-bearing milk from goat immunized against tetanus, published the first immunity curves. (2) Diphtheria. In diphtheria, clinical cases have been reported in which mothers recovering from the disease have apparently transmitted antitoxins to their infants (Kayser,¹⁹ and others). But it has been shown that a high percentage of infants from normal mothers show antitoxic properties in their sera after birth; therefore the interpretation of the above observations are open to question (Fischl and v. Wunschheim²⁰). Among animal observations, guinea-pigs (Wernicke,¹¹ Anderson²¹) and mare (Salomonsen and Madsen²²) have been shown to transmit immunity or antibodies against diphtheria to their young. (3) Pyocyanus. In some cases rabbits (Charrin and Gley²³), when immunized during the course of pregnancy, transmitted, to a certain degree, specific protective powers to their young. (4) Symptomatic anthrax. As mentioned, different workers have found that a certain degree of immunity is transmitted from the recently immunized mother to her young (Arloing, Cornevin and Thomas,⁴ Kitasato⁵). (5) *Vibrio* Nasik (vibriolysin). Wegelius²⁴ reports that goats and rabbits, when immunized either shortly before conception or during gestation, transmit the specific antibodies to their young.

c) Animal venoms. Fraser²⁵ states that a cat which was immunized against snake venom during the course of pregnancy gave birth to young which suckled mother; one kitten, when given two minimum lethal doses (subcutaneously) on the 57th day, showed marked resistance against the venom with only very slight symptoms,

from which it soon recovered, the other kitten on the 60th day was given three fatal doses of venom from which it failed to survive.

d Ferments. Morgenroth²⁶ immunized goats against rennin and found in a study of the milk that the specific antibody was present in considerable amounts. No example was found in the literature where studies were made upon the transmissibility of antibodies of this type from mother to young.

2. *Agglutinins*.—Including *a*) bacterial, and *b*) hemagglutinins. *a*) Bacterial agglutinins. (1) Typhoid. In view of the fact that much work has been done upon this group of agglutinins both (*a*) clinically (patients) and (*b*) experimentally (animals), a brief outline of the two subgroups will be given. (*a*) Among the clinical observations of possible intrauterine transmission of typhoid agglutinins from mother to fetus, a number of cases have been reported in which the mother aborted during the course of the disease. The body fluids from fetuses ranging in age from three to six months failed to show specific agglutinins (Etienne,²⁷ Charrier and Apert,²⁸ Dogliotti²⁹). However, Scholtz³⁰ reported a strong agglutinating reaction from fluids of a seven-months' fetus. In other cases (Schumacher,³¹ Stäubli,³² Mosse and Daunic³³) blood, taken from the fetal end of cord at birth (about or full term) of children born from mothers who had suffered during the course of pregnancy from typhoid, gave positive reactions. Other cases are reported (Castaigne,³⁴ Courmont and Cade³⁵) in which infants, taking the milk from mothers who were infected as late as two months (one case) after having given birth to young, also showed positive agglutinating sera. However, this was not always the case, since in a few instances (Kasel and Mann³⁶) the milks (2) were positive (1:25)—the blood agglutinated 1:50—but no reaction was given by the suckling's sera. The mothers in this series were infected 2–21 years before parturition. In another instance (Achard and Bensaude³⁷) a mother contracted the disease while nursing the child (age ?); the milk showed a low agglutinating value (1:10); the blood of the nursing was negative. In the other cases the milk from the mother when tested showed a positive reaction, with few exceptions. (*b*) The published studies upon animal experimentation add nothing further to the results already cited. Widal and Sicard³⁸ reported positive results in young rabbits born from a mother which was inoculated with typhoid bacillus six days before parturition. The heart blood taken from young at time of birth, however, was much weaker in agglutinating power than the mother's blood. Guinea-pigs actively immunized during pregnancy in most cases transmitted agglutinins to their young (Remlinger,¹³ Jurewitsch,³⁹ Stäubli⁴⁰). It is noteworthy that a goat which was highly immunized late in pregnancy gave birth to a kid whose blood showed no specific agglutinins (Schumacher³¹). This kid had not suckled before a blood sample was taken.

Among other organisms whose specific agglutinins have been reported as passing to the young from the actively immunized mothers, the following may be mentioned: (2) *B. tuberculosis*;^{*} specific agglutinins passed from infected mother to child (Lagriffoul and Pages⁴¹). (3) Cholera; specific agglutinins were transmitted from guinea-pigs immunized during pregnancy to the fetus or the young (Achard,⁴² Dieudonné⁴⁴). (4) *Proteus*; specific agglutinins were found in the serum of young guinea-pigs from immunized mothers (Achard⁴²).

Specific agglutinins for the above and other microorganisms have been demonstrated in the milk of immunized animals. But, as is evident, it does not follow that agglutinins are transmitted with any regularity from mother, either intrauterine or

* Figari (*Centralbl. f. Bakt.*, I, Ref., 1907, 39, p. 75), reports that hens fed upon tubercle bacillus preparations after a time showed specific agglutinins in their eggs.

through the milk to the offspring. For instance, a goat showing a reaction of 1:400 against anthrax failed to pass much agglutinin over to the still-born young; the kids' sera reacted in 1:10 dilution, which was much less than was normally present in the mother's blood before inoculation (Gengou⁴³). (b) Hemagglutinins. Kraus⁴⁴ reported that transmission of immune hemagglutinins did not take place in a kid born from a mother which had been immunized against red blood cells of sheep, but these immune bodies were demonstrated in the goat's milk. In infant's blood serum (cord) at time of birth, natural hemagglutinins were found to be less in amount than in the mother's serum (Halban,⁴⁵ Halban and Landsteiner⁴⁶), although there was much variation, depending upon the source of the test corpuscles.

3. *Precipitins*.—Specific precipitins against human blood serum have been reported by Merkel⁴⁷ in the serum of young rabbits (blood taken shortly after birth) from mothers which were immunized during the period of gestation. He considers that it was a case of placental transmission. Bertarelli⁴⁸ immunized two dogs by subcutaneous injections of horse serum and of cow serum respectively; later he found a small amount of precipitins in the milk of each but found none in the blood serum of their young after four weeks nursing.

4. *Cytolysins*.—a) Bacteriolysins. Normal bacteriolytic bodies have been frequently demonstrated in different milks; likewise specific bacteriolytic substances have been demonstrated in the milk of immunized animals (Kraus⁴⁹). No experimental work has been found demonstrating a transmission of specific bacteriolysins from the mother to the young. Clinically, several investigators have reported the relative bacteriolytic values of the mother's and infant's sera at the time of birth (Halban and Landsteiner⁴⁶), the infant's serum showing a less amount of natural bacteriolysins than the mother's serum.

b) Hemolysins. Several investigators have reported upon the transmission of specific hemolysins from immunized mothers to offspring either through the placenta or the milk. Immunized rabbits appear to transmit the hemolysins to the fetus through the placenta (Bullock,¹⁵ Bertino⁵⁰). Goats may or may not (Kraus,⁴⁴ Kreidl and Mandl⁵¹). In one case sheep failed to do so (Bertarelli⁴⁸). Natural hemolysins have been reported as being present in some cases in the serum of infants at birth. The question of the transmission of hemolysins will be taken up later in this paper, and be fully considered.

5. *Opsonins*.—Turton and Appleton⁵² compared the opsonic index of mother's and infant's sera in two cases (infants' ages, four and seven days) and found the infants' sera much lower than that of the mothers. Also they found, in comparative tests between the mother's serum and milk, that the latter was very much weaker in opsonins. It was not known whether the infants absorbed opsonin from the milk or not. This work has been in the main supported by other observers, both in human cases and by animal experimentation (v. Eisler and Sohms⁵³).

6. *Allergen*.—It has been shown that female guinea-pigs which have been sensitized by injections of proteins, as horse serum, bear young which are also hypersensitive to the same serum during the early weeks of life (Rosenau and Anderson,⁵⁴ Anderson,⁵⁵ Gay and Southard⁵⁶).

B. ANTIBODIES OF POORLY DIFFERENTIATED TYPES.

1. *Bacterial, intracellular toxins*.—As is well known, a large group of pathogenic bacteria do not excrete specific, soluble toxins to any extent, so their poisonous prop-

erties are supposed to be mostly due to cellular proteins or so-called intracellular toxins. Active immunity may be developed by recovery from the disease, or by experimental vaccination with killed or attenuated cultures. Not much is known concerning this type of immunity, but it is probably quite complex in nature and dependent upon a number of factors. However, it has been experimentally proved that a mother immunized with certain members of this group may transmit a specific immunity to her young; for example (1) anthrax (Chauveau³); (2) swine erysipelas (Ehrlich⁶); or (3) tuberculosis. Behring⁵⁷ observed that a 14-day-old calf, born from a highly immunized cow (bovine tuberculosis), possessed considerable immunity against the organism. Behring thought that this immunity was acquired by the calf through the milk rather than by intrauterine transmission. He suggested the milk of specifically immunized cows as a means of combating tuberculosis in man, either as a preventive or a therapeutic measure. (4) Dysentery. De Blasi⁵⁸ actively immunized a rabbit against the dysentery bacillus by three inoculations of killed cultures during the first 18 days after birth of young. The young (two) were given on the 10th day surely fatal doses of the dysentery culture. Both remained alive, whereas controls died.

2. *Spirilla*.—a) *Sp. obermeieri*. Animals may be actively immunized against this organism by further injections of spirillar blood following a recovery from the infection; others may be passively immunized by the injections of serum from such animals. Novy and Knapp⁵⁹ found young rats, born from infected mothers, immune when tested after a few weeks. They looked upon this as an active immunity, due to intrauterine infection, since the spirilla may penetrate the placenta and reach the fetus. Therefore it remains an open question if an active immunity (intrauterine infection), or passive immunity (placenta, milk) from mothers' antibodies occurs in the young.

b) *Spirochaeta pallida* (syphilis). It is questionable whether a complete immunity ever follows in syphilitic patients after a clinical recovery from the disease. With the introduction of the complement fixation test in the diagnosis of syphilis, it has been found that many apparently healthy children born from luetic mothers give a positive reaction, while others, nonsyphilitic, may contract the disease by suckling a diseased mother (Ricketts and Dick⁶⁰). This would prove that Profeta's Law* can no longer hold. It would appear that whatever immunity exists is slight. According to Thomsen,⁶¹ a number of observers besides himself have found complement fixing substances in the milk from luetic women. It would be of interest to know if an infant taking such milk from a luetic mother would absorb these bodies in sufficient quantity as to give a positive reacting serum. It is still questionable whether these complement fixing bodies are true antibodies, or simply metabolic products brought about by pathological changes in the body of the patient during the course of the disease.

3. *Protozoa*.—a) Piroplasmiasis (dog). Kleine and Möller⁶² showed that immunized dogs transmit some immunity to their young against this disease.

b) Rabies. This disease is only tentatively placed under this division since the question of the protozoan nature of the so-called Negri bodies is still under discussion. Hoegyes⁶³ was one of the first workers to point out that actively immunized female dogs may transmit some immunity to their offspring. Konradi⁶⁴ carried out similar experiments upon dogs and in several cases with positive results. The work of Remlinger⁶⁵ with rabbits also supported those results.

4. *Ultramicroscopic organisms*.—Under this heading may be placed a group of

* Profeta's Law assumed that a healthy child might be born from a luetic mother, and suckle the mother without contracting the disease.

contagious diseases which, on account of the constant failure to demonstrate definite specific microorganisms, has led many workers to consider the contagion in each case to be ultramicroscopic in size. (a) Sheep-pox (ovine). According to Burckhardt,² Richert immunized a large herd of sheep against ovine during the last six weeks of gestation. The lambs of these mothers were inoculated with ovine-lymph at the age of four to six weeks. None of them contracted the disease. A large number of controls showed pustules.

b) Foot and mouth disease. Loeffler⁶⁶ observed a calf born from a cow immunized against this disease, which proved to be immune against inoculations of the virus.

c) Small-pox and vaccinia. Since in the introductory portion of this paper small-pox has been briefly but sufficiently discussed, it will not be considered further. The work of Burckhardt² upon small-pox vaccine is of special interest since it was one of the first investigations upon the possible transfer of protective substances from mother to the child *in utero*. He vaccinated mothers during the last weeks of pregnancy, and then repeated the vaccinations upon the infants during the first days of their lives. He found in several that an apparent protection against the vaccinia had been acquired. Most of the control infants gave positive reactions. While these results were not conclusive, they offered suggestive data. In this connection it may prove of interest to note that in 1799, Hufeland (cited by Neumann⁶⁷) was perhaps the first to suggest that the milk might exert a protective power for the child when secreted by a mother who had recently recovered from the disease. In this class of diseases, as has been previously pointed out, it is always a question whether the young have been passively immunized from the mother's antibodies, or if an active immunization has taken place from having had the disease before birth.

III. DISCUSSION OF RESULTS.

In reviewing the literature on this subject, one is impressed by the broad scope of the studies, and the great amount of work which has been done. Even in the brief review just given, it is evident that the work on active immunity has been very productive of positive results. Also much work has been done by passively immunizing pregnant and nursing animals, but this has been purposely omitted in our résumé, since our studies cover only actively immunized mothers as influencing the offspring.

The possibility of an active intrauterine immunization on the part of the fetus must always be considered in connection with this type of experiment. Such may occur either through the passage of the living infectious agent or its products from the mother to the fetus. A distinction must be made between this condition and a passive immunization by means of the mother's antibodies through the placenta or through the milk to the young. In the diseases which are supposed to be due to ultramicroscopic organisms, the virus not infrequently seems to be able to pass the placenta and

affect the fetus *in utero*. Where immune offspring are born from mothers which have passed through such a disease during pregnancy, caution must be exercised against accepting this as a true passive transmission of immunity (*in utero*) from the mother. To this we must add that animal experiments with certain living spirilla must be considered in the same light, since they may also pass the placenta and affect the fetus.

Most bacteria do not pass the intact placenta except under extraordinary conditions. Further, recent work (besides our own) would indicate that the fetus, or the very young animal, possesses only feeble reactive powers as to antibody production, when attempts have been made to actively immunize by means of prepared antigens. As shown by the foregoing review, fluids from fetuses aborted early in pregnancy by infected mothers usually gave negative results when tested for specific antibodies. Judging from these investigations we should expect the best results in the young from animals which have been actively immunized either against dead cells or toxins during the latter part of gestation. Even where such conditions were observed, positive results did not follow in every case. In part, the negative results may be accounted for by differences in experimental conditions, such as nature of antigen used, degree of active immunization attained, time of immunization relative to birth of young, character of resulting antibody, time when blood from offspring was first taken, etc. Further, many workers failed to make any distinction between the rôle played by placental or by milk transmission of antibodies; in many cases the blood of young was not drawn until after having the mother's colostrum and milk. In fact, blood samples in some instances were not taken until weeks after the birth of the young. Much of the literature cited in the above review covers very careful and painstaking investigations which have been carried out upon this subject. These studies prove that when the mother has been properly immunized during gestation and shows a high degree of immunity at the time of birth of young, some of this immunity (antibodies) may be passively transmitted to her young. This occurs either through the placenta before birth, or by means of the milk immediately after birth.

IV. REPORT UPON ADDITIONAL WORK CONCERNING TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING.

1. *Introductory remarks.*—As pointed out in the foregoing résumé, the passive transmission of antibodies to the offspring is practically established. This seems to hold for almost all classes of infectious organisms where a true active immunity (formation of antibodies) takes place in the mother's body. But the relative importance of the exact parts played by the two separate means of transmission—placenta or milk—has not been so fully decided. Some workers maintain that the placenta plays the greater rôle, while another but smaller group holds that antibodies do not pass the intact placenta. More work upon this subject, bearing in mind this phase of the question, seems desirable.

A large group of infectious diseases are induced by organisms which do not produce extracellular toxins (in culture) sufficient in amount or of such a nature that antitoxins can be produced by animal immunization. Recognizing the importance of this group of endotoxin-producing organisms, and their significance in disease, it was thought worth while to take up analogous studies which might throw more light upon the transmission of immunity to offspring. Since if by vaccinating the mother cautiously during pregnancy, the offspring also becomes immunized, a means is offered by which the young may be protected against those infections (pneumococcus, etc.) to which they are particularly susceptible during the first weeks of life. Up to the present time, we have no curative serum which offers much hope in these conditions. Active immunization (by vaccines) of the newly born against these organisms offers little encouragement and, in fact, may be followed by harmful sequelae. Not much is known concerning the nature of the immunity induced by actively immunizing animals against this type of organism. But perhaps in so far as the serum of such animals is concerned, the contained antibodies are quite complex in nature, and include antitoxins, bacteriolysins, opsonins, etc. Also there seems to be evidence that such a serum contains substances of which we know little, other than that they produce favorable results, exclusive of the bodies already mentioned. Since cytolysis must be one of the important factors in this type of

immunity, the cytolytins may act as an index in carrying out experimental studies upon such immune sera.

As mentioned in the review, little has been done directly upon the bacteriolytic antibodies concerning their transmission from mother to offspring, but bacteriolysins have been found in milk from different animals. Most workers who have carried out studies along cytolytic lines have used lysins against red blood cells—the hemolysins.

In my own work, which is to follow, this means was adopted, as it appeared most advantageous. For instance, the test can be carried out completely in test tubes, and, under proper technic, with a high degree of accuracy. Also, test animals, as necessary in diphtheria or tetanus antitoxin work, can be entirely eliminated. As compared with bacteriolytic work the hemolytic tests are much more rapidly performed and probably more easily graded.

2. *Review of work reported upon transmission of hemolytic antibodies.*—After a careful search through the literature I find that several investigators have already reported work concerning the transmission of hemolysins from mother to offspring. However, some of this work escaped my attention until after my own work was well under way. Although briefly mentioned in the previous general review, these reports will now be considered more fully.

Kraus,⁴⁴ in 1901, reported his work upon the possible transmission of immune hemagglutinins and immune hemolysins from mother to offspring. He found that rabbits immunized by repeated subcutaneous injections of defibrinated dog's blood showed in their milk specific hemagglutinins, but these bodies were not carried over to the blood of the suckling. For the study of hemolysins, he immunized goats by injecting with sheep blood. No hemolysin could be found in the milk, so he concluded that these immune bodies were not excreted by the mammary glands, although the blood serum of the same animal showed quite definite amounts of the hemolysin. However, he found that the young born from one of the immunized goats showed a low degree of immunity (hemolysin) which was transient. Kraus thought that this was acquired before birth, and considered it a passive immunization from an actively immunized mother. He gives no definite data concerning the hemolytic tests of the serum but apparently he added no normal complementing serum, but depended upon the small amount of complement which was present in the immune serum used in the test. As is well known, goat serum is weak in hemolytic complement, and therefore will not bring out the highest efficiency of the contained immune hemolysin, even when present in large amounts. As a result, his data show the hemolytic sera to be of very low value. But in the milk tests he added normal goat serum, as complement, since he thought it possible that the milk glands might not let the complement through,

consequently no activation of the immune body could take place in case it were present. In view of negative results, he concluded that immune body was not present in the milk. He did other experiments with goats' milk which indicated that it was deficient in complement.

Following this, Bulloch,¹⁵ in 1902, took up the study of transmission of hemolysin from parent to offspring, using rabbits immunized against blood cells of the ox. He considered not only the rôle of the immunized mother, but also that of the father from the standpoint of heredity, and obtained results which, on the whole, supported the early work of Ehrlich. But of special interest to us are his findings concerning the transmission of specific hemolysin from mother to the young. When the blood injections were given to the gravid animals, pregnancy was interrupted, but the fetal fluids were found strongly hemolytic. In those cases in which the injections were made prior to conception, the young were reared, and in one case the inactivated serum of the young was equal to that of the mother in hemolytic power, while the milk was only one-third as active as the mother's serum. In another case the serum of the young, which was found dead on the day of birth, was one-half the value of that of the mother, while the milk was about one-fourth of that value. Bulloch agreed with Kraus that no complement passed out with the milk,* but showed in opposition to Kraus that hemolytic immune body is excreted by the milk glands. He used fresh guinea-pig serum as complement in all of his tests.

Kreidl and Mandl¹⁶ (1904), in a preliminary report, stated that goats injected with beef blood might lead to a passive immunization of the fetus, but from their tests concluded that in the majority of cases, the specific hemolysins do not pass from mother to fetus through the placenta. They considered individual variations to be responsible for the lack of uniformity in their results. No definite experimental data are given in this report. They also consider the transmission of immune hemolysins from the fetus over to the mother, but as their results are not fully conclusive, they will not be discussed in this connection.

Bertino¹⁰ (1905) states that in rabbits, when the immunization of mother has taken place before conception, the lysins do not pass over to the fetus, nor to young during the suckling period, but when the immunization is carried out during pregnancy, there is a transmission of the specific antibodies to the fetus. Since the original article was not available these results are quoted from an abstract (*Folia Haematologica*, 1905, 2, p. 624), and I am unable to give further details.

Bertarelli¹⁸ (1906) reports that he found a small amount of specific hemolysins in the milk of a sheep immunized against hen's blood, but found none in the blood of the young. This immunization was carried out by repeated injections of the blood during the last weeks of pregnancy and the first weeks after birth of lambs.

Considering the report just presented, it would appear that hemolysins may be transmitted through the placenta to the fetus *in utero*, especially in the examples cited by Bulloch. But objections may be raised as to the validity of the findings from aborted fetuses, since in those cases a placental lesion could exist which

* Lane-Claypon (*Jour. Path. and Bact.*, 1908-9, 13, p. 34) claims to have demonstrated complement in fresh milk (in about 1/10 value of the individual's serum) as well as natural immune bodies, by the addition of the "ox colloid" of Bordet. Others have also shown the presence of the hemolytic complement in milk (Pfaundler and Moro; *Ztschr. f. exper. Path. u. Therap.*, 1907, 4, p. 451).

would permit a direct connection between the maternal and fetal circulation. Apparently the ox blood injected seemed to exert a toxic action upon rabbits. It would be difficult to exclude the influence of absorption of hemolysins from milk previous to time blood samples were taken from young, in some of the cases. The question of animal species may enter largely in determining the results. The meager data available in Bertino's work upon rabbits would support the statement of Bulloch. Perhaps larger animals, as goats or sheep, do not permit this class of antibodies to pass the placenta so readily as some of the smaller animals.

Another important point, which must be considered in such experiments involving specific hemolysins, is the presence of natural hemolysins (for the blood used) in the blood serum of the mother, and their development in the body of the fetus, or possible passage over from the mother's circulation. Much variation in this respect may exist between different individuals of the same species. It is necessary carefully to control adult serum as to content of natural immune bodies against the particular blood to be used for immunizing purposes, before any injections are made.

3. *Choice of experimental animals and outline of technic.*—In the present work goats were selected for experimental animals since they are more highly developed, rugged, and withstand experimental handling (injections, bleedings, etc.) very readily, and usually give a bountiful supply of milk. Sheep corpuscles were used for immunizing purposes; their advantage being that goat serum seldom shows the presence of more than a small content of natural hemolysins, if any, against these corpuscles. Goats readily respond to the blood injections by specific hemolysin production. This immunity may be raised to a high degree by repeated injections of the blood, while practically no toxic action is exerted upon the animal. Moreover, only a small amount of hemagglutinins are formed by goats against sheep corpuscles; hemagglutinins when present in large amounts, in a hemolytic serum, cause a marked disturbance in the hemolytic reaction.

In my experiments, the technic followed was similar in all cases, and may be briefly outlined as follows:

1. Preparation of sheep's blood for injection, or for hemolytic test: Blood was drawn from the jugular vein of normal sheep by puncturing the skin and vessel wall

with a large hollow needle and collecting the flow in a sterile bottle containing a coil of wire. It was defibrinated by shaking, then placed in tubes, centrifuged, the serum removed, and then the corpuscles were washed three to four times in 0.9 per cent NaCl solution until they were practically free from all serum.

2. The injections of blood in these experiments were all made subcutaneously. If the volume was very large, it was divided and injected into the animal in several places.

3. Collections of blood samples. Bleedings were made from the jugular vein. In case of adult, a hollow needle was used, but from kids, a few c.c. were drawn by means of a hypodermic syringe. In each case blood was placed in a test tube and allowed to clot out. When the serum had separated, it was removed and placed in small sterile bottles.

4. Milk samples had 10 per cent by volume of 1:1,000 formaldehyde in 0.9 per cent NaCl solution added. Preliminary tests showed that this strength of formaldehyde solution exerted an antiseptic action, while it had little if any influence upon hemolytic antibodies in milk. The milk samples were then placed in bottles for storage. Both serum and milk samples were stored in ice-box where they were kept until the entire series covering the experiment were collected. Tests were done with the fluid portion which was removed from under the cream layer, after this had separated. The first colostrum, which was heavy and tenacious, failed to separate a cream layer, so was used as a whole.

5. When a full series of samples of sera and milks for a given experiment were collected, all were tested at the same time, thereby using the same blood suspension, complement, technic, etc., which made the results much more comparable. Preliminary tests were first made to get relative values of the samples. The serum became "inactivated" by aging; no heat was applied in any case. The ordinary test tube method for the determination of specific hemolytic immune body* was employed.

For complement, in the earlier experiments, fresh rabbit serum (0.20 c.c. per tube) was used; later, fresh serum from guinea-pigs (0.10 c.c. per tube) was used. This proved to be more efficient, and was used in all tests which are cited except when otherwise noted.

The scheme followed for the tests was to arrange a series of test tubes for each sample; the serum or milk which was to be tested was first accurately measured in decreasing amounts directly from sample or from the proper dilution in physiological (0.9 per cent) salt solution dilution, into a series of test tubes. Then physiological salt solution was added to bring the volume of each tube up to 1.0 c.c. To this was added the given volume of complementing serum, then 2 c.c. of 7.5 per cent suspension of sheep corpuscles. Immediately the tubes with the mixtures were thoroughly shaken, then placed in the incubator (36°-37° C.). After one hour they were removed, again thoroughly shaken and placed in the ice-box until any remaining, intact corpuscles settled to the bottom of the test tube. After the corpuscles had sedimented, a transparent fluid remained above, varying from a deep red color to a water white, depending upon the presence or absence of hemolysis. The results of the tests were read by making equi-colorimetric readings throughout the various series of samples by com-

*The term "hemolytic immune body" is used in this report as a more suitable equivalent than "substance sensibilisatrice" (Bordet), or "intermediary body" "amboceptor" (Ehrlich) for the biological reaction product of the organism against injected alien blood cells.

paring with a "standard color" tube which was made by "taking" a given volume of the blood corpuscles in distilled water.

4. *Groups of experiments.*—For convenience the experiments will be considered under four groups; the first two cover the main tests upon the transmission of hemolysins from the mother to the young, while the remaining groups cover certain tests which have a general bearing upon the subject.

In the first group the female goats were actively immunized against sheep corpuscles by the method outlined. Each experiment was a comparison of the relative content of specific hemolysins in the mother's serum, the serum of her own young, and of her colostrum or milk. No "cross nursing or feeding" of young was undertaken. The tests are not given in perfect chronological order, but each experiment will be given a number for reference purposes, and these will run consecutively throughout the report. In order to avoid repetition, typical experiments will be reported in full; others falling under the same head will be briefly described.

a) Active immunization of the adult before birth of the young, either during the period of gestation, or before conception had taken place.

SERIES I. PRELIMINARY EXPERIMENTS.

To obtain data concerning the possible transmission of hemolysins from mother goats to their young, preliminary experiments were undertaken. Since the results proved instructive from several standpoints, a part of the data will be submitted. In this series, pregnant animals were immunized during the last weeks of gestation or immediately before the birth of the young. Blood samples were taken from the adults before the injections were started, and then at short intervals during the course of immunization. On day of birth of the young, blood samples from the mother and young, and a sample of the mother's colostrum were taken. Following this, samples were taken at intervals for two and a half to five weeks or even longer.

Experiment 1. Goat 4 (normal; pregnant).—During the course of nine days this animal received six subcutaneous injections (increasing doses) of washed sheep corpuscles—a total of about 400 c.c. On the morning of the 12th day after the last injection it was found that she had given birth to two normal kids; one was removed from the mother about noon, and placed on cow's milk although it had undoubtedly

freely suckled its mother. Late in the afternoon blood samples were taken from each kid. During the same forenoon, both blood and colostrum-milk samples had been taken from the mother. Test samples from each were taken at intervals for over two weeks following the birth of the young. Comparative tests (regular technic, 0.20 c.c. rabbit serum for complement) were made upon all of the samples at the same time and the following results were given:

Time	Mother's Serum	Colostrum-Milk	Kid "A" Serum (Mother's Milk)	Kid "B" Serum (Cow's Milk)
Day of birth of young	0.06 c.c.*	0.02-0.03 c.c.	0.035-0.04 c.c.	0.045 c.c.
1st day after birth	0.055 "	0.20-0.30 "	0.04 "	0.05 "
2d " " "	0.055 "	Traces	0.045 "	0.08 "
4th " " "	0.06 "	0	0.06 "	0.08 "
6th " " "	0.035 "	0	0.08 "	0.09 "
10th " " "	0.047 "	0.20 c.c.	0.10 "	0.175 "
14th " " "	0.075 "	0	0.15 "	0.20 "

*When comparisons are made between the samples it must be understood that the potency value is inverse to the quantity value. The numbers indicate the volume of each sample which was required to produce a given degree (constant) hemolysis in that test.

Further tests showed that the antibody content of the blood in both kids continued to fall, more or less in parallel. The adult's serum showed its highest value on the sixth day after the birth of the young (18th day after the last injection of blood), then gradually declined. The milk showed transiently (eighth to 10th day) demonstrable amounts of antibodies, following shortly after the adult serum showed its greatest concentration in antibodies.

Experiment 2. Goat 14 (normal; pregnant).—Two subcutaneous injections of washed sheep corpuscles were given on consecutive days: first day, 125 c.c.; second day, about 140 c.c. Twenty-one days after the last injection, the mother was found in the morning with one normal kid which was allowed to remain with her. At noon blood samples were taken from both and also a sample of the mother's colostrum-milk. The kid had suckled and stomach seemed full at time the bleeding was made. During the course of immunization, blood samples were collected from the mother, and this was continued with the addition of milk samples at intervals for some time after parturition. Likewise, blood samples were taken from the kid, and finally all samples were tested at the same time to get the relative hemolytic values. The adult's serum showed its maximum hemolytic value (0.0075 c.c.) 13 days before birth of young. From this high value it rapidly receded until on the day of birth of young it gave a comparative value of only 0.035 c.c. It remained fairly constant at the same level for about one week, then gradually fell. The colostrum-milk showed a value of 0.05-0.06 c.c. on day of birth of young; the antibodies rapidly disappeared from the milk so that by the second day after the birth the test failed to show their presence. The kid's serum from blood samples taken on day of birth showed a value of 0.017 c.c., while a sample taken a few days later showed about twice this value, then gradually receded. This would lead us to believe that gastro-intestinal absorption of hemolysins from the milk is an important factor in the very young animal. The first blood sample was taken from the kid a few hours after birth and although it had freely fed, the absorption of antibodies was still incomplete but the assimilation from the digestive tract continued after this time, as was indicated by higher value of its blood serum taken later. This question will be taken up again and more fully discussed.

Experiment 3. Goat (normal; pregnant).—This animal was given subcutaneous injections of washed sheep corpuscles spaced three days apart—three in all—100 c.c., 75 c.c., and 75 c.c. A kid was born during the evening or night following the last injection; it was allowed to remain with the mother. The mother's serum showed no definite immune bodies on day of birth of the kid. Two days later blood samples from both were drawn and a milk sample was taken from the mother; this was repeated on alternate days for several weeks. All were tested at the same time, using 0.20 c.c. of rabbit's serum for complement. The mother's serum showed its highest value in specific hemolysins on the 10th day after last injection of blood cells, then gradually receded from this point. The milk showed a low antibody content; the mother's serum and milk reached their highest concentration simultaneously (serum 0.0035 c.c., milk 0.20–0.25 c.c.). The kid's serum showed questionable traces of specific hemolysin on the fourth and sixth days after birth but none later, although the blood serum was watched for several weeks. This test shows that an immunity to erythrocytes arising during the period of lactation has an influence upon the milk, but that the antibody content is less than 1 per cent of that of blood. Further, a kid at this age must receive much higher quantities of antibodies through the milk, in order to absorb a sufficient quantity to be easily demonstrable in its blood serum. This will be more fully supported by results of other tests which are to follow.

These preliminary tests indicate that mothers actively immunized against the erythrocytes during the latter part of pregnancy can transmit these antibodies to the offspring. But when the immunization has progressed only slightly, that is, no antibodies are demonstrable in the blood until after the birth of the young, the young fail to show more than a trace of antibodies in their blood. Presumably these are acquired through the milk. Probably in all cases this transmission was passive, and took place either through the placenta or the colostrum-milk, or both. I was led to think from these early tests that of the two factors each was about equal in importance. It was found that the colostrum-milk was rich in specific antibodies when the females were immunized before parturition. Also, the kids showed a rise of antibody content in their blood, as they took and absorbed this colostrum, which rapidly reached a maximum and then decreased slowly, as in passive immunization. Further, it was observed that an animal which received first blood injection only a few days before and the last on day of birth of young, did not influence the offspring to any appreciable extent; the milk showed a very low antibody content.

SERIES 2. SPECIAL EXPERIMENTS.

A series of experiments were undertaken by which the relative importance of the two factors—placenta and colostrum-milk—in

Experiment 7. Goat ♀ (pregnant).—It may be of interest to record in this connection an observation made on another goat which might be classed with this series. The mother showed signs of oncoming labor during the late afternoon, so both blood and colostrum samples were taken. Next morning it was found that the animal had given birth to two large (weight four lbs. each), apparently fully developed young which were dead. Nothing in particular was shown on autopsy excepting some subcutaneous edema in each; some bloody fluid was found, in the thoracic cavity, which was removed and saved for test; the heart and large vessels were fairly well filled with partially clotted blood which was removed and the serum collected; lungs, nondilated, on section showed nothing unusual; the other organs appeared normal. Possibly a large bleeding (between 300–400 c.c.) which was taken from the mother one week before, might have had some influence upon the welfare of the young. The weight of the adult, on day following the birth of young, was 83 lbs. Upon centrifuging the fluid from the thorax, and the blood from heart and great vessels, it was found that in one kid the fluids came out quite clear, while those from the other were rather deeply colored by the presence of hemoglobin. We shall therefore consider the tests made upon the clearer fluids from the one kid. The following comparative values were shown: Mother's serum, 0.0025 c.c.; Colostrum, 0.0015–0.0020 c.c.; Kid—the fluids from the blood and from the thorax failed to show a definite hemolysis in amounts up to 0.20 c.c.; a very slight tint was observed but it is questionable if this were due to specific antibodies or traces of hemoglobin in the sample.

This adult was unique in the sense that she seemed to retain a very high antibody content in her blood long after the last blood cell injection. The last blood injection was given about six months (170 days) before birth of the young. Most animals lose their antibodies much more quickly; few show them in appreciable quantities after six months. This animal was immunized before conception had taken place, since the period of gestation in goats is stated to be five months. If the hemolytic immune bodies passed through the placenta to the fetus in this case, the amount was very small.

From the experimental evidence given in this last series of experiments it would appear that the colostrum plays a greater rôle in the transmission of hemolytic immunity to the offspring than the placenta—the latter probably transmits little if any appreciable amount of hemolytic immune body, to the fetus *in utero*.

b) Active immunization of the adult following immediately or shortly after birth of young.—A number of workers have succeeded in highly immunizing female animals, during the period of lactation, against toxins and getting the antibody in the milk in a relatively high concentration. Since it has been proved that the milk is an important factor in transmitting antibodies to the suckling during the first days of life through gastro-intestinal absorption, it was thought worth while to undertake similar experiments at later periods in connection with our studies.

SERIES 3. ANIMALS NORMAL AT TIME OF PARTURITION.

Experiment 8. Goat 8 (normal). Preliminary test.—This animal gave birth to one normal kid which was allowed to remain with and suckle mother. Blood samples were taken from the mother and kid, then the mother received a subcutaneous injection of 75 c.c. of sheep corpuscles. Following this, 100 c.c. corpuscles were given daily for three consecutive days—a total of 375 c.c. Blood samples were drawn from the mother and kid on alternate days for two weeks, after which samples were taken every third day for some time longer. In this experiment no milk samples were taken during the first weeks as the supply was scant; the serum samples were tested at the same time using 0.20 c.c. rabbit serum per tube for complement. It was found that antibodies did not appear in definite amounts in the mother's serum until five days after the last blood injection, then they rapidly increased until the 11th day, when the highest concentration (0.0035 c.c.) was found; the antibodies slowly declined until on the 46th day after last injection the value had fallen to 0.037 c.c. Serum samples from the kid when tested in amounts as high as 0.5 c.c. failed to show positive results. It is questionable if any appreciable amount of hemolysin was excreted in the milk when the mother's serum was at its greatest concentration. If so, the kid at that age failed to absorb (unchanged) a sufficient quantity to give a definite hemolytic reaction in the test.

Experiments 9 and 10.—Similar experiments were performed with goats 4 and 5, with the exception that milk samples were taken throughout and tested at the same time as the blood serum samples of the respective mother and kid. In neither case did we succeed in getting very high immunity in the mother animals, and it was found that an appreciable amount of antibody failed to pass over in the milk. Naturally the serum of the kids did not show antibodies.

These experiments proved quite unsatisfactory. It appears that an exceedingly high immunization of the female is necessary before a fairly high antibody content occurs in the milk. In neither of the two goats last mentioned was the degree of immunization sufficient to cause a demonstrable overflow of antibodies in their milk.

An obvious objection to the active immunization of normal females immediately after birth of young is the necessary delay before the production of antibodies in high concentration in the mother's body, and their appearance in the milk. In the meanwhile the young animal becomes more mature; its digestive powers are more pronounced, and probably anatomical changes occur in the gastro-intestinal mucosa which retard or prevent the absorption of the unchanged antibodies.

SERIES 4. ANIMALS PARTLY IMMUNIZED AT TIME OF PARTURITION.

In order partly to overcome certain objections to which attention has just been directed, animals which had been previously

immunized and still showed a low antibody content in their blood at the time of birth of the young, were reimmunized by more blood injections following parturition. In this way it was hoped that a high immunity could be induced in the mother, and more quickly than was possible in the strictly normal animals, and that the milk would either remain high or increase in immune body value. Possibly kids receiving such milk in quantity during the first weeks of life would be able to absorb the immune body unchanged, and as a result their sera would remain about constant or even show some decided rise in the hemolysins. In opposition, other factors enter, such as (a) animal growth (increase in body fluid), (b) loss through blood samples withdrawn, and the (c) metabolic destruction of antibodies. Therefore, such experiments should be well controlled.

Experiment II. Goat 6 (previously immunized).—Found in the forenoon with normal twins, and as they had suckled, only a small sample of milk could be obtained from the mother. Both remained with mother until late in the afternoon. The mother was given a large injection of washed sheep corpuscles the same evening.* The blood injections were repeated every second day until four in all were given—a total of 425 c.c. Blood samples (adult and kid) and milk were taken for several weeks. All were tested simultaneously. The results of the test may be tabulated as follows:

Time	Mother's Serum	Milk	Kid "A" Serum (Mother's Milk)	Kid "B" Serum (Cow's Milk)
Day of birth of young.	0.0080 c.c.	0.05-0.06 c.c.	0.002 c.c.	0.0035 c.c.
2d day after birth of young. .	0.0077 "	0.27 c.c.	0.002 "	0.0030 "
4th " " " " " "	0.0040 "	0.16 "	0.003 "	0.0035 "
6th " " " " " "	0.0020 "	0.08 "	0.003 "	0.0040 "
10th " " " " " "	0.0009 "	0.05 "	0.004 "	0.005 "
12th " " " " " "	0.00008 "	0.045 "	0.0042 "	0.005 "
16th " " " " " "	0.0012 "	0.05 "	0.006 "	0.007 "
22d " " " " " "	0.0017 "	0.15 + "	0.0078 "	0.019 "
28th " " " " " "	0.0022 "	0.15 + "	0.009 "	0.03 "
34th " " " " " "	0.0027 "	0.18 "	0.015 "	0.03 "
40th " " " " " "	0.003 "	0.27 "	0.022 "	0.045 "

(When 45 days old, Kid "A" weighed 14½ lbs., and Kid "B," 17 lbs.)

As evident from the above data, the kid which was with the mother and getting a bountiful supply of milk containing large amounts of immune body, showed in its blood serum an antibody content practically running parallel with that of a control (brother) which was fed upon cow's milk after the first day following birth.

* At the same time this animal received injections of killed cultures of the colon bacillus in small but increasing doses on days alternating with the blood injections. These injections were continued over two weeks without any harmful effects in so far as noted.

The ratio of weight between the two kids probably ran roughly parallel throughout the test. So it would appear, at least in this instance, that not many of the antibodies pass over unchanged to the circulation from the gastro-intestinal tract of a suckling animal after the first days following birth.

This experiment would suggest that even when we can get an animal highly immunized and the milk shows the presence of considerable antibodies, hardly an appreciable amount is absorbed unchanged from the digestive tract by the suckling after the first days of life. These results were supported by other observations, but in experiments where a control twin was not available.

c) Active immunization of newly born or older kids against foreign blood cells.

SERIES 5. BLOOD INJECTIONS INTO KIDS.

In connection with our studies where mothers received very large injections of blood during gestation, especially in the very last weeks, we must consider the possibility of soluble antigens passing the placenta to the fetus. Bang and Forssman⁶⁸ state that ox corpuscles gave an ether soluble fraction which possessed antigenic properties when injected into suitable animals. It is possible, therefore, that in the body also similar soluble products are liberated in quantity and eventually reach the blood stream from the large subcutaneous blood injections. Hektoen and Carlson⁶⁹ have shown by transfusion experiments with dogs, which had been previously injected (intravenous) with small amounts of goat's blood cells, that after several hours the antigen is fixed in the body of the injected animal, and not transmitted in sufficient quantity to the normal recipient to cause specific antibody production in the latter. But when large blood injections were given to a donor, then later a direct transfusion was made to a normal animal, the recipient as well as donor produced antibodies in due course of time. This showed that the body's ability to fix the formed antigen was limited. Therefore the possibility exists that, from large subcutaneous blood injections into gravid goats, soluble antigen might eventually reach the fetus. The question arises, If the fetus receives antigen shortly before birth, has it the power to react and

form antibodies, or may it retain the antigen and form antibodies shortly after birth? Any attempt directly to inject the fetus* *in utero* is necessarily surrounded by numerous technical difficulties. Moreover, the results thus derived would be justly subject to criticism. A few experiments were carried out on kids at different periods after their birth to test their reactive ability against sheep erythrocytes. The results may throw some light upon this side of the immunity problem.

Experiment 12. Kid 96 B (newly born).—This kid was a twin born from a mother which had been immunized against sheep corpuscles, over five months before parturition. The mother's blood taken six days before birth of the young showed a trace of hemolytic immune body in 0.10 c.c. serum, while a sample taken on the day of birth failed to show any hemolysin in like amount of serum. The colostrum on day of birth of young showed a small amount of immune body in 0.10 c.c., but the milk on the following day was negative. A blood sample was taken from the kid before it was allowed to suckle, then it was placed with the mother. The same evening 12.5 c.c. of sheep corpuscles were injected into the kid subcutaneously and the next afternoon another injection of the same amount of corpuscles was given. Blood samples (eight) were taken at intervals during the 15 days following the last injection. On testing it was found that 0.10 c.c. serum from blood samples taken the second and third days after birth showed a small amount of hemolysin, but this practically disappeared and soon showed only as a trace. Since the kid's blood sample taken before suckling showed no hemolytic action in the same amount, one must look to the colostrum as a source of its antibody on second and third days. The tests of samples, covering two weeks following last blood injection, failed to show more than a trace of hemolysis at any time, but perhaps this trace was slightly greater at the end of the second week. Adults usually show specific hemolysin production by the end of the first week after injection, and often this is at a maximum on the ninth to 12th day. We must conclude that the young in this case produced hemolysins only to a very slight extent (minute quantity) in response to the injection of sheep blood corpuscles in relatively large amounts.

Experiment 13. Kid 15 B (10 days old).—This kid was also born from an immunized mother, but failed to show any appreciable amount of specific hemolysins in its blood serum at the end of the first week after birth. On the 10th day after its birth the kid was given a subcutaneous injection of 10 c.c. of sheep blood corpuscles and an equal dose was given in the same way on the 12th day. Samples were collected at intervals for three weeks after the last injection. By the test,† all were negative (0.10 c.c.) until the ninth day, when a slight trace of hemolysin was shown; on the 12th day this was somewhat increased, a definite but slight hemolysis; 16th day, questionable trace; 20th day, 0.10 c.c. gave negative results. This would indicate that a slight but transient immunity reaction took place against the sheep blood cells.

* Kreidl and Mandl (*loc. cit.*) attempted injection of the fetus (goats) *in utero*, but were unsuccessful in the outcome in practically every case.

† Used the regular technic excepting 2.0 c.c. of a 5 per cent instead of a 7.5 per cent suspension of sheep erythrocytes were added per tube.

Experiment 14. 68 B (18 days old).—The mother of this kid had been immunized against sheep blood cells, some time previously. It was removed from the mother on day of birth and fed during the first days upon goat's milk and then later upon cow's milk. On the 18th day after its birth, 20 c.c. sheep blood cells were injected into it subcutaneously and two days later an equal volume was given in the same way. Blood samples were taken during three weeks following the last injection. Testing by the regular technic, it was found that 0.10 c.c. of serum showed a trace of antibody, on the 18th day after birth (first injection of blood). Probably it got colostrum, which contained immune body, on day of birth. These antibodies quickly disappeared and further samples were negative in amount tested, until six days after last blood injection, when a slight trace again appeared and gradually increased so that on the 12th day after the last injection of blood, a slight but definite hemolysis was produced by 0.10 c.c. of serum; this had again practically disappeared within a week. This experiment indicates that only a very slight immunity reaction occurs in a kid after it has reached the age of 18 to 20 days.

If we are permitted to draw any conclusions from these few experiments we should say that the newly born animal has only slight ability to form hemolysins during the first days following birth. Consequently we cannot assume that the fetus *in utero* has greater power, even if antigen should reach it through the placenta. But, on the other hand, it is more probable that the fetus has even lesser power to form immunity reaction product than the newly born. This seems to be a property which is only gradually acquired by the growing young, as they adjust themselves to their environment in the external world. The work of several investigators (Schkarin,⁷⁰ Moll⁷¹) would seem to substantiate the view that the organism of very young infants or animals have only a slight ability for antibody production.

d) Gastro-intestinal absorption of antibodies.

SERIES 6. FEEDING OF HEMOLYTIC SERUM TO NEWLY BORN KIDS.

As apparent from the data submitted, the newly born kid readily absorbs in an unchanged condition the specific hemolytic immune body from colostrum through the gastro-intestinal tract. These immune bodies do not appear in the kid's blood serum unless present in considerable amounts in the ingested colostrum. However, the amount in colostrum-milk may be comparatively low and still cause a decided increase in hemolytic value of the suckling's blood, if the total volume taken is large. The question suggested itself, Was this free absorption of immune body taken through the milk-colostrum peculiar to the combination? Or, are the immune

bodies readily absorbed by the newly born kid, when fed hemolytic serum, irrespective of the nature of the medium in which they are present? As we had an opportunity, this question was put to test in two cases by feeding newly born kids with homologous serum (goat) which contained specific hemolysins. The kids were taken away from the mothers immediately after birth and were never allowed to suckle. Owing to the lack of animals at the time this investigation was in progress, exact quantitative experiments upon this question, unfortunately, could not be continued. The following results may prove to be of some interest in that direction:

Experiment 15. Kid 112 B (newly born, weight 1,350 gms.).—A twin, which was not allowed to suckle mother, was removed immediately after birth and a blood sample was drawn. An immunized goat (15) was bled at once, the blood defibrinated and centrifuged to remove all corpuscles. About 30 c.c. of this supernatant serum was fed to the kid and no other feeding was made until the next day, when it was given cow's milk, which was continued from that time on. Unfortunately on testing this serum it was found to be of much lower value than suspected; test showed that 0.02 c.c. produced, however, a well marked hemolysis when tested by the regular technic, with the exception that 2.0 c.c. of 5 per cent instead of 7.5 per cent sheep blood suspension was used.

Samples of blood were taken upon four consecutive days after the feeding of the immune serum. When these samples (including the sample taken from the kid before feeding) were tested, in the manner just mentioned, none showed hemolysis in amounts of 0.15–0.20 c.c. If one-third of the antibodies were absorbed (unchanged) one would expect the test to give at least a slightly positive reaction in the volume of kid's serum which was tested. It follows that the absorption of unchanged antibodies from the digestive tract in this animal was not pronounced.

Experiment 16. Kid 97 B (newly born, weight 2,535 gms.).—Immediately after birth this kid was removed without being permitted to suckle. A blood sample was taken, then it was fed by pipette 15 c.c. of specific immune serum (goat 15) which had been kept under good condition, in freezing ice-box, for almost four months. This hemolytic serum, tested shortly after the feeding experiment, was of such potency that 0.002 c.c. almost completely hemolysed 3 c.c. of 5 per cent sheep blood suspension (0.10 c.c. guinea-pig serum for complement), while 0.001 c.c. gave a definite hemolysis. About one hour after this feeding the kid was given 150 c.c. of cow's milk. No further feeding was made until the next morning, when it was put upon cow's milk regularly. Blood samples were drawn daily at first, then at longer intervals, covering in all 15 days' observation. The tests were carried out upon these samples by the same technic (amount of blood samples, complement, and volume fluid) employed in the preceding experiment (Kid 112 B). It was found that the sample taken before the feeding of hemolytic serum was negative in 0.10 c.c.; the day following the immune serum feeding, 0.10 c.c. serum showed a well marked action; the second day after, the same amount of serum showed less hemolytic action; the hemolytic power gradually diminishing until, on the 13th and 15th days after the serum feeding, no action was shown by 0.10 c.c. This experiment showed that considerable absorption (approximately

10 per cent) of the unchanged hemolytic serum from the digestive tract took place, and appeared in the general circulation of the young animal. Recognizing the fact that no definite quantitative conclusions can be drawn from as few as two experiments, no more can be said than that these experiments suggest that antibody absorption while taking place is relatively meager when hemolytic sera are fed to newly born kids.

5. *General discussion of results.*—Before attempting to draw any definite conclusions from the above experimental data, it may be well to consider more fully some of the findings, and the related questions. In this brief discussion, not only our own results, but the correlated results of other workers, will be considered. The experiments clearly demonstrate that goats, when highly immunized against sheep erythrocytes during the latter part of gestation, transmit the specific antibodies to their sucklings. Apparently the placenta plays a very small part in this transmission, but the principal rôle is played by the antibodies which are present in the colostrum-milk and are readily absorbed from the digestive tract when taken by the newly born offspring. In those cases where the young born from immune mothers were prevented from taking the colostrum-milk until after a blood sample had been drawn, tests failed to show the presence of hemolysins in the kid's blood serum in sufficient amount to be appreciable by the method used. So we must conclude that the fetus *in utero* acquires from the actively immunized mother little if any hemolytic immune-body. We must therefore direct our attention particularly to the antibody bearing colostrum-milk, and the gastro-intestinal absorption of antibodies by the young.

Observations upon the colostrum, taken at time of parturition, and before the young were permitted to suckle, proved to be very interesting. When compared with the same animal's blood serum, taken at the same time and tested in parallel, it was sometimes found to be considerably higher in immune-body value, in some instances reaching a value two to three times greater than the blood serum. The published data of other workers would indicate that this occurs not infrequently where animals are immunized, some time before birth of young, against various antigens (Stäubli,^{32, 80} Wegelius²⁴). But particular attention has not been given to its importance. The question arises, Is the high hemolytic

value of the colostrum in these cases due to (1) accumulated specific antibodies formed primarily in the mammary gland, or (2) a storage of antibody removed from the blood stream, or (3) are these antibodies present only in a concentration equivalent to that of the other body fluids but are augmented in action by other substances present in the colostrum, e.g., substances analogous to the "bovine colloid" of Bordet and Gay⁷²?

As yet we know little concerning which tissues or organs form the hemolysins. The theory has been advanced, with some experimental support, that the blood-forming organs are the principal factors in the production of antibodies (Hektoen⁷³). According to Hektoen,^{69, 74} the blood itself, and probably the subcutaneous tissues at site of injection of blood corpuscles, do not fix the antigen and produce hemolysins to any appreciable extent. If this view concerning the formation of antibodies is accepted, little reason exists to suppose that the hemolysins are being formed to any extent in the mammary gland. No experimental proof has been brought forward showing that the cells of secreting glands produce antibodies. So, on the whole, little evidence exists which would support the first possibility.

Probably the second possibility mentioned is the true explanation of what actually occurs. In the absence of any direct experimental work on this particular question, certain facts may be considered, and a hypothesis deduced which will support the view. The quantitative relationship between the protein constituents of normal colostrum, and normal milk, in conjunction with known facts, must be considered. In the absence of full chemical data upon goat's colostrum and milk, the data upon analogous products from the cow must be used. According to König's⁷⁵ tables, the composition of goat's and cow's milk is very similar. Hoppe-Seyler-Thierfelder⁷⁶ states that colostrum is probably somewhat poorer in casein than milk, but very much richer in albumin and globulin. König's tables show practically the same general results with the exception of the casein, which he gives in a higher percentage for the average colostrum (4 per cent) than in the milk (3 per cent); he states as the average for coagulable protein (albumin and globulin) in colostrum, 13.6 per cent—for milk (albumin)

0.53 per cent. The colostrum contains an especially high content of globulin (Eichelberg⁷⁷), while the milk contains only traces of this protein. At once we are struck by the great extremes of protein content existing between the colostrum and the milk from the cow. But it is also noticeable that cow's colostrum and milk do not show this great quantitative variation between their casein contents. Some observers report that the average casein content is about the same in colostrum and later milk. Casein is considered to be a nucleo-albumin, which is elaborated by the glandular epithelium and according to Mandel⁷⁸ probably originates in the gland cells from a breaking down of the nucleo-protein. So the casein content of the colostrum or milk may be used as an index of the secretory activity of the gland. The lacto-albumin which is present in the milk is also probably elaborated in the gland cells, and, with the casein, constitutes the normal milk proteins. The trace of lacto-globulin present in later milk has the properties of the sero-globulin, and probably is simply excreted by the gland cells by removal from the blood stream in an unchanged condition. In support of this supposition the work of Bauer⁷⁹ may be quoted. He prepared anti-sera by the injections of serum, colostrum, and milk. By a refined complement fixation test he was able clearly to differentiate blood serum and milk from the same animal. But when the anti-serum from cow colostrum was used, he found on testing that the highest specificity was shown against the same colostrum ($\frac{1}{100,000}$) but also he had a fixation with cow's milk ($\frac{1}{10,000}$) and ox serum ($\frac{1}{10,000}$). These results showed that in colostrum there is present, not only the special milk proteins, but also those peculiar to the same animal's blood serum. His experiments would indicate also that the milk proteins were a specialized product of the milk glands. From both the purely chemical and the biological reaction standpoint, it would appear that the proteins of colostrum and milk are somewhat different in qualitative character and in origin. The colostrum contains, in addition to those proteins of the normal milk, blood serum proteins, especially globulin, in considerable quantity.

It is a well known fact that during the active immunization of an animal its blood serum shows a decided increase in its protein

content.* Also it is equally well known that antibodies themselves, if not protein in character, are closely associated with the proteins in particular the serum globulins. Since we have seen that globulins are present in considerable quantity in colostrum, and that the biological test showed that colostrum contained serum protein while milk did not, we can assume that this is a seroglobulin, as only a trace of globulin is found in normal milk. The high concentration of antibodies in the colostrum might be explained by the excretion of serum proteins (globulin, etc.) by the glandular epithelium, and incidentally the specific antibodies which are probably closely associated. The antibody value, if the hypothesis is true, would parallel the protein concentration in the colostrum.

Stäubli^{32, 80} made similar observations upon typhoid agglutinin-colostrum and blood serum. He found that the colostrum from a clinical case, as well as animal experiments, showed much higher agglutinin content than the blood serum at the same time. He thought that perhaps the milk glands took up the antibody from the blood and returned the water, thus causing a concentration; and also that a certain parallelism existed between the coagulable proteins (lacto-albumin, lacto-globulin) and the agglutinins. He considered the gland cells as playing an important *active* rôle in the formation of agglutinins, and that the high value of colostrum was influenced by the destruction of those cells.

The possibility that substances may be present in colostrum, which might augment the action of the hemolysins, offers some points of theoretical interest. But the supposition is hardly tenable as an explanation, since the dilution of the colostrum was so great in many instances that a "colloidal action" would be negligible. Other chemical substances present would also suffer in the same way. These substances would also appear in the normal colostrum drawn from the animal before immunization, but in no case did we ever get a hemolysis from such a colostrum, in the

*Ledingham (*Jour. Hyg.*, 1907, 7, p. 65) reported quantitative protein changes in serum of a goat during an immunization against diphtheria toxin. The total protein in the normal serum before injection of toxin was 5.867 per cent; the maximum reached during the course of immunization was 8.555 per cent, then gradually diminished. Gibson and Banzhaf (*Jour. Exper. Med.*, 1910, 12, p. 411) never found the total proteins to rise above 10.52 per cent during the active immunization of horses (11 animals) against diphtheria or tetanus toxins.

presence of rabbits or guinea-pig complement, even when used in large quantities.

Before leaving this side of the question we wish to say that it is highly desirable that further work should be carried out along these particular lines.

The gastro-intestinal absorption of unchanged antibodies from immune serum has received attention ever since the introduction of serum therapy. Escherich⁸¹ attempted to passively immunize children by giving antidiphtheritic serum *per os*. He reported that infants of the first months showed a slight rise of antitoxin in their blood, but in older children he met with no success. Laboratory animals failed to absorb appreciable amounts of antitoxin, even when fed in relatively large quantities (Carrière⁸²). The work of the v. Behring school (Behring¹⁷, Ransom⁵⁷, Roemer⁸³) confirmed the findings that adult animals under normal conditions do not absorb antitoxic serum (heterologous) in an unchanged condition, but the newly born animals do so readily from the mother's milk (homologous). They attempted to explain this on the basis of the differences between the anatomical structure of the gastro-intestinal mucosa in the newly born and the grown (Disse⁸⁴), but these claims have not been verified. At the present time certain other factors are considered very important in this process. Salge⁸⁵ fed antidiphtheritic serum to infants, but states that no antitoxin passed over to the blood. He observed that infants getting antitoxin through the milk of the nurse readily absorbed it. He considered this difference to be due to homologous antibodies in the milk which were easily absorbed, while the heterologous antidiphtheritic serum was not assimilated in an unaltered state. Salge,⁸⁶ in order to test this point, fed infants for several weeks with goat milk (heterologous) drawn from immunized animals and showing considerable antitoxic value, but was unable to demonstrate the presence of antibodies in the blood of the infants. The work of Bertarelli⁸⁷ and others would tend to support the same view, although this point is not fully settled.

In my experiments it was seen that newly born kids readily absorbed hemolysins when taking the mother's colostrum-milk containing these bodies. Also in one case, in which the homologous

hemolytic serum (highly potent) was fed, it was absorbed in considerable amounts but probably not so readily as the colostrum-milk hemolysins. Uffenheimer⁸⁸ in his extensive work upon gastrointestinal absorption (bacteria, proteins, antibodies) touched upon the absorption of hemolysins. He fed guinea-pigs, newly born or during the first days of life, considerable amounts of hemolytic serum derived from rabbits immunized against guinea-pig blood cells. He concluded that none of the hemolysins was absorbed, since the young animals gave no evidence of intoxication (hemoglobulinuria, decrease in number of blood cells, etc.). Much smaller doses of the same serum when injected into controls caused their death.

The reports of several investigators (Ganghofner u. Langer,⁸⁹ Uffenheimer,⁸⁸ Roemer u. Much⁹⁰), upon the question of the gastrointestinal absorption of unchanged proteins, etc., by young animals, call attention to various factors which influence the absorption, such as age, overfeeding of proteins, the nature and source of the proteins, digestive powers, irritation or lesions of the intestinal mucosa, species of animal, individual variation, etc. In considering the possibility of immunizing the young animal through the digestive tract, the above factors must be kept in mind.

The failure to transmit immune bodies to the suckling when the mother is immunized after its birth, in all probability, is chiefly due to two reasons: first, the milk immune bodies do not appear in the milk at any time in very great quantities; while secondly, the time of their appearance is past the critical age point where the young are able to absorb the hemolysin (unchanged) to any extent.

When immunization against sheep corpuscles was carried out in goats during the period of lactation, the antibody content of the milk when compared with that of the serum was never greater than 1:45-50. Similar observations upon goats immunized against diphtheria and tetanus showed that the milk contained relatively greater amounts of antitoxin when compared with the serum, than shown in the above ratio for hemolysins (Ehrlich u. Wassermann⁹¹).

All observers are agreed in the conclusion that the newly born animal (or infant) absorbs from the digestive tract unaltered antibodies most readily when given immediately after birth. The

most effective means of supplying the antibodies to the young is through the mother's colostrum-milk (homologous); probably next in importance is the homologous serum, and finally the antibody milk or serum from some other species.

It follows from our observations that of all factors the colostrum plays the most important rôle in transmitting antibodies from the actively immunized mother to the young. Even under normal conditions, it is reasonable to suppose that protective substances are concentrated in the colostrum from the mother's body fluids and may be in part absorbed by the infant after the first feedings. Consequently it seems of highest importance that the newly born child should, during the first days of life, receive the colostrum-milk, unless it is contra-indicated for some special reason. Perhaps the natural resistance which many infants show against many infectious diseases may be partially explained on the basis that the child is passively immunized by anti-substances excreted through the immune mother's colostrum and milk, which are absorbed by nurslings during the early days of life. Such a view gains strength from the observations of Moro,⁹² who found that the blood serum from breast-fed infants showed greater bactericidal and hemolytic powers than that from infants who were artificially fed upon cow's milk. The practical application, of adding to the mother's immunity by vaccines during pregnancy, would undoubtedly be attended with risk of disturbing the natural course of pregnancy, and should not be entered into unless further investigation demonstrates its safety.

6. *Summary*.—To summarize briefly the principal results of the foregoing experiments, it was found that:

1. Goats actively immunized against sheep blood corpuscles during gestation passively transmitted the specific hemolysin to their young.

2. The colostrum was the chief agent in bringing about the passive immunization of the suckling. Sucklings which got the colostrum and first milk rapidly acquired a relatively high antibody content in their blood, which was well retained.

3. When the immunization was done during the period of gestation the colostrum contained a high content of specific

hemolysin, often much higher than the adult's serum, at time of parturition.

4. The hemolytic antibodies rapidly disappeared from the milk after the mother had been suckled by the young.

5. The blood taken from the newly born before they were permitted the antibody colostrum showed no appreciable amount of hemolysin by the test used. The placenta played a minor rôle in the passage of hemolysins to young before birth, practically negligible in most cases.

6. Mother goats, actively immunized against sheep-blood corpuscles immediately after birth of their young, failed to transmit any demonstrable immunity to their suckling young.

7. The milk, in some cases, contained no demonstrable hemolysins, but in others showed fairly large amounts.

8. Apparently a very high degree of immunity is necessary before appreciable amounts of antibodies are excreted through the milk.

9. Older sucklings apparently did not absorb the antibodies in an unchanged condition.

10. The young animals (kids) did not respond to any extent in production of hemolysins following subcutaneous injections of foreign blood cells (sheep).

In conclusion, I wish to express my indebtedness to Dr. William H. Park, Director of Laboratories, Department of Health, for many helpful suggestions in connection with the above work. I am also indebted to Drs. Paul Bartholow, and Dr. Maria Grund, for valuable assistance.

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THE PROPERTIES OF DESICCATED RABIES VIRUS AND ITS USE IN ANTIRABIC IMMUNIZATION.*†

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The preservation of rabies virus by desiccation has been reported by Harris and Shackell,¹ who, employing the method described by one of them (Shackell),² succeeded in maintaining the infectivity of this material for several months. The method consisted in freezing the brain or cord with a mixture of salt and ice and drying this *in vacuo* at a temperature below -10° C.

The object of this paper is to describe a modification of the original method, and to record the results of a quantitative determination of the amount of infectivity remaining in the dry material, and the value of this material in the study of the principles of anti-rabic immunization. I present also some observations on the essential difference between desiccation at the ordinary temperature and desiccation of thoroughly frozen material at a temperature below 0° C.

Tests of the material frozen by means of salt and ice as described in the first paper showed the amount of virulence remaining after complete desiccation to be from 1 to 2 per cent of the original quantity. Experiments undertaken to find a means of preserving a larger quantity of the infective agent resulted in the observation that *the more thoroughly and rapidly the material is frozen, the greater will be the amount of virulence remaining after desiccation*. When rabies virus is frozen with carbon dioxide snow and then dried *in vacuo* without being allowed to melt, from 30 to 50 per cent of the original infectivity is preserved. Allowing for a 60 to 70 per cent loss in weight, due to the extraction of the water, the dried cord contains as much virulence per milligram as the fresh.

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¹ *Jour. Infect. Dis.*, 1911, 8, p. 47.

² *Am. Jour. Physiol.*, 1909, 24, p. 325.

The method in detail is as follows: The brain or cord is ground in a porcelain mortar, with the addition of water, drop by drop, until a thick, smooth paste is formed. Carbon dioxide snow is then collected from a tank in the ordinary manner and is added in small amounts to the paste, which should be stirred thoroughly meanwhile to prevent the material from freezing in a solid mass. Freezing occurs rapidly and, when complete, the material is very brittle and easily reducible to a fine powder.

Carbon dioxide snow may be easily obtained from the ordinary tank of liquid carbon dioxide by allowing this to flow rapidly into a bag made of several layers of sterile towels. If the towels be wet beforehand the bag will freeze into a solid container and the snow may be emptied from time to time in any desired quantity.

During the pulverization more snow should be added from time to time to prevent thawing. When the material is thoroughly pulverized, it is transferred to a small beaker with an excess of snow and placed in the bottom of a Scheibler's vacuum jar which has previously been half immersed in a mixture of salt and ice and become thoroughly cold. A beaker of sulfuric acid is then placed on wire gauze in the upper part of the jar in such a manner that there is free access of air between the frozen material and the sulfuric acid. The acid is placed in the upper part because, if placed below, it soon freezes at the low temperature. The vacuum should measure less than 2 mm. of mercury.

During the desiccation the temperature should not be allowed to rise above -15° C. The jar should be rotated gently several times daily to mix the absorbed water and the acid. Unless this is done, the time required for the absorption of the water vapor by the acid will be unduly prolonged. A single brain will become thoroughly dry in from 36 to 48 hours. More material will require a longer time.

A satisfactory container for the ice and salt is easily made by surrounding a five-gallon earthenware jar with a thick layer of mineral wool. When this has once become cold throughout, the salt and ice will maintain a temperature below -15° C. for 36 to 48 hours. Should the amount of material to be dehydrated contain more water than will be readily absorbed by the sulfuric acid, the

vacuum jar may be reopened and a fresh beaker of concentrated sulfuric acid changed for the diluted. During this transfer the temperature of the virus must not be allowed to rise and, to avoid this, the jar should remain semisubmerged in the cold mixture. In the same way a fresh supply of salt and ice may replace the melted brine, provided steps be taken to prevent a rise in the temperature of the jar meanwhile.

The object in thoroughly pulverizing the virus is twofold. It results in a more complete mixture, so that all parts contain an equal amount of virulence. Secondly, it permits of more rapid drying and an easy transfer into smaller containers for subsequent tests.

To avoid any absorption of moisture, it is preferable to transfer the dry powder from the beaker to small glass tubes and seal the end in the flame. This transfer is effected in a moisture-free atmosphere by covering the top of the beaker with rubber dam held in place by adhesive strips. A small puncture is made in this sheet large enough to admit the tube, and through this the tubes are inserted and filled. From 20 to 100 mg. is a convenient amount to put into each tube. If the diameter of the tube is 4 mm. each millimeter of powder will weigh approximately 2 mg.

A number of intracerebral injections were made with seven different lots of dried virus. In all of these the amount of desiccated *cord* necessary to produce paresis in a rabbit within six to seven days was not more than $1/50$ of a milligram. The minimal lethal dose of desiccated *brain* is $1/200$ of a milligram (0.00005 gm.). Tests were not made until the material had been thoroughly dry for at least seven days.

Although unknown to us at the time the first work was done, Vansteenberg¹ had succeeded some years earlier in preserving the infectivity of rabies virus by desiccation *in vacuo*. He noted in 1903 that if rabic material were ground into a thin pulp, spread out in a *very thin layer* and desiccated in a rapidly produced vacuum, its infectivity could be preserved for many months, provided the resulting powder was kept in a dark, cool place, free from moisture. Others repeated this experiment, but agreed that the amount of

¹ *Compt. rend. Soc. de biol.*, 1903, 55, p. 1046.

virulence remaining was very small, and that the material was valueless for immunizing purposes (Stimson).¹

Harvey and McKendrick² believe that the loss of virulence in cords dried by Pasteur's method is practically proportionate to the loss of water. In the first paper we stated: "Our work leads us to believe that it is the *method* of extracting the water which results in alteration or destruction of virulence, and not the extraction of water *per se*. To state it differently, slow desiccation attenuates and destroys the virus directly by reason of the concentration of salts and other substances which are in solution in the brain and cord. The action is therefore, in essence, a chemical one. Desiccation of frozen material avoids any concentration of those intra- and extracellular salts or substances which are at the ordinary temperature in solution. With this method absolute dryness proceeds cell by cell from the surface. If this proposition be true, Vansteenberg's and Marie's successes and failures are easy of explanation. These authors emphasize the fact that the cord must be spread in a very thin layer and the vacuum produced very rapidly. A vacuum rapidly produced will freeze a small quantity of water in a bell jar. The success of these writers, in our opinion, depended upon the freezing of their thinly spread material and its drying without concentration. Further support is given to our hypothesis by the fact that exposure of thoroughly dried material to ordinary air destroys its virulence completely within a few hours. The absorbed atmospheric moisture is, in this case, sufficient to redissolve some of the salts and other soluble materials in a most concentrated state and destroy by chemical means the inclosed virus."

The present investigation has, it seems, established the correctness of the view expressed at that time. Partial freezing in the first work resulted in the conservation of only 1 or 2 per cent of virulence. Even the freezing with carbon dioxide snow does not result in complete solidification since the cryoscopic temperature is estimated to be between -50° and -100° C. But if this temperature (below -50° C.) could be maintained until drying is complete, one should get a powder more virulent than that which

¹ *Bull. No. 65, Hygienic Lab., U.S. Public Health and Marine Hospital Service, Washington, 1910* p. 46.

² *Theory and Practice in Anti-rabic Immunization, Calcutta, 1907.*

I have secured by drying at -18°C . (salt and ice). Material frozen with liquid air and kept at that temperature until dry should contain practically all of the original infectivity.

To ascertain the physical effect of desiccation at low temperature, pieces of brain, kidney, liver, etc., have been frozen and dried *in toto*, fixed in absolute alcohol, transferred to xylol and imbedded in paraffin. These organs when frozen with salt and ice and dried at -18° show so much cell distortion as to be scarcely recognizable. When frozen with carbon dioxide and dried, as above described, the cells appear considerably altered and shriveled, with here and there an island on the surface fairly well preserved. When pieces of these organs are dropped into a beaker of liquid air and then dried, the sections show but slight morphological changes in the cells. Cell boundary and shape, nucleus and nucleolus are all preserved with surprisingly small changes. The alterations on the surface of the block are less than those in the deeper lying layers. These sections are striking illustrations of what small physical alterations occur in the drying of frozen material, and offer visible evidence of the extensive cellular alteration which results from osmosis and chemical change when dehydration is carried on at a temperature higher than the cryoscopic point.

Desiccated material kept in a cool, dark place (8 to 10°C .), free from moisture, loses its virulence very slowly. The following tests show the loss of virulence in one lot sealed and kept in an ice-box (8 to 10°C .).

R-184. Brains of three rabbits removed October 26. Placed on ice 24 hours, then ground up. Frozen with carbon dioxide snow and desiccated from October 27 to November 1, 1911. Transferred to tubes November 9 and sealed *in vacuo*.

November 10:	2 rabbits (A and B)	inoculated with	0.02 mg.
2 "	C and D)	" "	0.04 mg.
2 "	E and F)	" "	0.20 mg.

Four of these (A, C, E, and F) were paretic on the 6th, one (D) on the 7th, and one (B) on the 8th day.

February 5:	1 rabbit	inoculated with	0.05 mg.
1 "	"	"	0.10 mg.

The rabbits showed symptoms on the 6th and 7th day.

March 21 (1912) (142 days after complete desiccation):

1 rabbit	inoculated with	0.10 mg.
1 "	"	1.0 mg.

Both rabbits paretic on the 7th day.

This material possesses, after a preservation *in vacuo* for 142 days, a virulence greater than that which Harvey and McKendrick found in two-day cords dried according to Pasteur's method.

Other lots of material kept in a desiccating jar in the presence of sulfuric acid show the same rate of loss in infectivity as when kept in sealed tubes. When kept in the light or in the presence of phosphoric anhydride the virulence is lost more rapidly. The loss at room temperature has not as yet been determined.

Liquid air has been used as a preliminary freezing medium, and while the desiccated powder was slightly more virulent (M.L.D. of brain = 0.000004 gm.) the results are of more scientific interest than practical value.

Tests of the immunizing properties of this material have been made on dogs, rabbits, and human beings. Several dogs, which had received severe lacerations and deep bites about the head by dogs known to be rabid, have been immunized and deep injections of a large amount of rabid dog's brain have been given to immunized dogs. The number of immunizing doses has varied from four to seven and the amount injected has varied from 10 to 50 mg. per dose. None of these animals has shown symptoms of rabies. Some were killed after one to two months and their brains found to be non-infective.

About 50 rabbits have been immunized and tested. These received from five to 14 immunizing doses of from 10 to 30 mg. each. In no instance has the injection of this material produced symptoms of rabies either in dogs or rabbits. Some of the rabbits have shown a partial immunization to subsequent subdural injection of street virus, and some have shown a complete immunity to intraocular injections of street virus.

In those that died, the period of incubation of the disease was lengthened from 18 days (control) to 40 and 60 days. Inasmuch as all investigators are agreed that complete immunity is established in the rabbit only after a prolonged treatment, I did not anticipate that these animals would survive the tests. The object of these tests was more to establish the innocuousness of the subcutaneous injections of the material than to produce a complete immunity in rabbits.

There are at the present time a number of dogs and rabbits under observation, and when these and other tests have been completed a subsequent paper will give a detailed and tabulated result of this part of the work.

The injections into human beings were not made until it had been shown that as many as 1,000 to 3,000 times a dose fatal to a rabbit (intracerebral) could be injected subcutaneously into dogs and rabbits without ill result. The first to receive injections was one of the laboratory staff. He received seven daily injections each of which contained 1,000 times the infective dose for rabbits. Since that time the material has been used in the treatment of 17 persons bitten by rabid dogs and cats.

This brings me to a discussion of the value of this material in settling some of the differences of opinion and practice in antirabic treatment. In some institutes treatment is begun with 14- or 10-, or 6- or 5-day cord. Some use Högyes' method, others follow the method of Ferran. There are in fact almost as many modifications as there are antirabic institutes.

Nitsch and others have shown that the amount of infective material varies throughout the cord, and that the cervical region is five times as virulent as the lumbar. It is evident from this that the amount of infective material, or, to use a more exact term, the number of lethal rabbit doses, injected from day to day is variable and does not depend entirely upon the amount of cord used. Even with the so-called exact method of Högyes, the variation must be considerable unless the same portion of the cord is chosen from day to day.

If the conclusion of Harvey and McKendrick that "the immunizing power of a given portion of rabies cord is a function of the unkilld remnant of rabies virus which is contained in that cord," is true, one should be able to find out with mathematical certainty how many minimum infective doses will produce a definite degree of immunity.

The very gradual and constant rate with which infectivity diminishes in desiccated material enables one to ascertain the number of M.L.D. administered in each injection. In these experiments I have adopted as a standardizing unit the smallest quantity

which, when injected intracerebrally in a full grown rabbit, will produce paresis within six or seven days. In determining the degree of immunity established, it is also necessary to know the exact amount of street virus injected. For this purpose the infectivity of a desiccated brain (street virus) is being standardized.

The following problems present themselves in this connection and are offered here in the hope that others may be induced to work along this line.

To what extent does immunization depend upon the number of infective units injected?

Are a few large doses as efficient as the large number of injections of varying strength given according to the methods of Pasteur and Högyes?

Is there any danger in commencing the treatment with large doses, e.g., 1,000 units, and repeating this daily?

Have all strains of fixed virus approximately the same virulence?

Are all strains equally innocuous to dogs and rabbits and human beings when administered subcutaneously in the usual manner of treatment?

The questions above outlined are by no means new. They have, in fact, been asked many times before. I repeat them here, however, because I believe they may be more easily decided by the use of a virus of known and relatively permanent infectivity, i.e., a standardized virus.

SUMMARY.

Rabic material may be completely desiccated without destruction of virulence, provided the dehydration takes place at a low temperature.

The lower the temperature, the greater will be the amount of virulence preserved.

The desiccated virus contains per weight as much infectivity as the fresh virus.

The loss of virulence is so slow that the material may be standardized, permitting an accuracy of dosage hitherto impossible.

The unit is the smallest amount which, when injected intra-

cerebrally into a full grown rabbit, will produce paresis on the seventh day.

The use of desiccated virus in antirabic immunization of animals and persons offers many advantages over other methods.

I wish to express my obligation to Mr. L. F. Shackell, whose enthusiasm led to the undertaking of the problem, and whose advice has been of much assistance in working out the details of the present method.

CALCIUM SALTS AND THE ONSET OF LABOR.*

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In a recent communication¹ we have shown that the colostrum of the normal cow, as well as that of a cow ill with parturient paresis, contains a substance or substances capable of bringing about abortion in pregnant guinea-pigs. It was also shown that the substance or substances in the fresh, normal colostrum which excite the pregnant guinea-pig to premature labor withstand heating to boiling for a short time. We reached the conclusion, therefore, that in this respect the exciting substance of the colostrum resembles the hormones, and the further conclusion would seem to be justified from the experimental evidence obtained in our studies that the onset of labor in the pregnant female is brought about by the internal secretions of the mammary gland. For reasons which we hope to discuss more fully in a subsequent communication, it occurred to us that perhaps calcium salts might prove to be an important factor in the onset of labor.

In this connection it is of interest to observe that W. Blair Bell and Paintland Hick,² in their admirable studies on calcium metabolism and the periodic variation of the calcium content of the blood during the menstrual period in woman, foresaw a causal connection between the fall of calcium in the systemic blood and its corresponding increase in the menstrual discharge, and the onset of labor in the pregnant female. They proved conclusively that there is an excretion of calcium during the menstrual period which is coincident with the fall of calcium in the blood. They have also shown that calcium salts exert a definite influence upon the uterine contractions, with the slight but definite rise of blood pressure. According to these authors a slow, forcible, and rythmical contraction of the uterus is produced by the action of calcium salts similar in all respects to that seen in labor; and while they were

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¹ *Jour. Infec. Dis.*, 1912, 10, p. 244.

² *Brit. Med. Jour.*, 1909, 1, p. 517.

unable to show definitely that labor is produced by the action of calcium salts in the blood their work certainly indicates such a possibility, in that they have shown that the uterine secretions are rich in calcium salts. They are therefore inclined to the belief that while possibly other substances of a hormonal nature may act in conjunction with calcium salts, the latter are probably specific in bringing on the rhythmical, expulsive contractions of the menstruating uterus and the uterus in labor. Later Halford,¹ from observations on the remedial effect of calcium salts in pre-eclamptic conditions in woman also arrived at the conclusion that calcium salts may be responsible for the onset of labor in the pregnant female. So far as we have been able to ascertain, however, no one has yet succeeded in proving experimentally that calcium salts can bring on the onset of labor. That calcium salts can cause the premature onset of labor in pregnant guinea-pigs is evident from the following experiments.

In our previous work on the effect of colostrum on pregnant guinea-pigs we had already tested the effect of normal physiological salt solution (.85 per cent NaCl) by way of control. As was to be expected, such a solution was without visible effect. Accordingly, in the tests about to be described, the several solutions of calcium and other salts employed were made approximately isotonic with a normal saline, containing 0.9 per cent NaCl. We have also included one sodium, potassium, and magnesium salt by way of comparison and control. The calcium salts employed were the lactate and glycerophosphate (Merck's preparations). The sodium, potassium and magnesium lactates were prepared by neutralizing the required amounts of N 1 lactic acid with the calculated amounts of the several bases. All of the solutions employed were exactly neutral to litmus. The additional data are given in the following:

EXPERIMENT I. *Calcium lactate*.—A sterile solution of calcium lactate was prepared containing 3.25 per cent of $\text{Ca}(\text{C}_3\text{H}_5\text{O}_3)_2 \cdot 5\text{H}_2\text{O}$. On analysis this was found by Mr. O. M. Shedd of this laboratory to contain 0.5910 gm. CaO in 100 c.c. This solution was tested on a pregnant guinea-pig with the following result: A healthy female guinea-pig, weighing 640 gms., in the fifth to seventh week of pregnancy, received by intraperitoneal injection 9 c.c. of the above solution of calcium lactate, at 4 P.M., March 29, 1912. The pig suffered no discomfort from the injection and showed no discomfort during the next two hours. On the following morning, viz., at 9 A.M. on

¹ *Australas. M. Gaz.*, 1909, 28, p. 595.

March 30, 1912, it was found that this pig had given premature birth to three fetuses, weighing together 140 gms., and measuring 9, 9.5, and 9 cm., respectively. The three fetuses were covered with hair and one of them was covered with membranes. The cotyledons were still found attached to the three fetuses. The mother pig had passed 40 c.c. of urine during the night and now weighed 450 gms. She remained well during the next few days and on April 9 was returned to the piggery.

EXPERIMENT 2. *Calcium glycerophosphate*.—A sterile solution of calcium glycerophosphate (Merck) was prepared containing 1.5634 per cent of $C_3H_5(OH)_2CaPO_4H_2O$. On analysis this solution was found by Mr. Shedd to contain 0.3840 gm. CaO in 100 c.c. A healthy female guinea-pig, weighing 670 gms., in the fifth to seventh week of pregnancy, received by intraperitoneal injection 9 c.c. of the above solution of calcium glycerophosphate, at 4 P.M., March 29, 1912. For an hour following the injection this pig showed no discomfort. She then urinated and began to show considerable discomfort, apparently in abdomen. During this interval she rolled around on her sides and lay for short intervals more or less flat on her back and was observed to strain down on the pelvic contents. At the end of the second hour after the injection of the calcium glycerophosphate she was more comfortable and quiet and apparently sleeping. She was then left for the night. At 9 A.M., March 30, 1912, it was found that this pig had given premature birth to one fetus, 11.5 cm. long and weighing 90 gms. The fetus was covered with hair and had one cotyledon attached. The mother had passed 40 c.c. of urine and now weighed 540 gms. This pig remained well during the next few days, although she lost in weight. At the time of her return to the piggery on April 9 she weighed 450 gms. The urine of the two pigs that had aborted under the influence of glycerophosphate and lactate of calcium showed a marked increase in the output of calcium in the urine, during the seven days following the abortion. During this interval the calcium in the urine was found by Mr. Shedd to increase gradually from .016 to .126 per cent CaO. Before the injection of the calcium salts their urine contained only .009 per cent CaO.

EXPERIMENT 3. *Sodium lactate*.—A sterile solution of sodium lactate was prepared containing 1.723 gms. $Na(C_3H_5O_3)$, in 100 c.c., by neutralizing 15.4 c.c. N/1 lactic acid with the required amount of pure sodium hydroxide. A healthy female guinea-pig, in the fifth to seventh week of pregnancy, weighing 950 gms., received by intraperitoneal injection 9 c.c. of the above solution of sodium lactate, at noon on April 1, 1912. The pig struggled a little during the injection but 20 minutes later was comfortable and eating cabbage. On the morning of April 2 this pig was found well. No evidence of premature labor and no diarrhea. At 10:45 A.M., April 3, this pig seemed entirely well and was still pregnant; weight 890 gms. At this time this pig received, by intraperitoneal injection, 2 c.c. of normal human colostrum, made up to 9 c.c. with sterile normal salt solution. The pig suffered no discomfort and was eating cabbage in 15 minutes after the injection. April 4, pig seemed well; no evidence of premature labor. During the night of April 4 she gave premature birth to three fetuses, with three cotyledons attached, weighing altogether 194 gms., and measuring 9.5, 10, and 11 cm. in length, respectively. All of the fetuses were covered with hair and one was still covered with membranes. This pig remained well during the next few days and on April 9 was returned to piggery, weighing 590 gms.

EXPERIMENT 4. *Potassium lactate*.—A sterile solution of potassium lactate was prepared containing 1.9727 gms. $K(C_3H_5O_3)$ in 100 c.c., by neutralizing 15.4 c.c. N/1 lactic acid with the required amount of pure potassium hydroxide. A healthy female

guinea-pig, weighing 970 gms., and in the fifth to seventh week of pregnancy, received by intraperitoneal injection 9 c.c. of the above solution of potassium lactate, at 12:45 P.M., April 1, 1912. She struggled a little during the injection, but at 12:30 she was quiet and comfortable. She remained normal during the entire afternoon of April 1. On the morning of April 2 she was found to have a marked diarrhea, otherwise normal. No evidence of premature labor. On April 3 the pig was well, the diarrhea having subsided. Stools formed. No evidence of premature labor. On April 4 this pig was well—no evidence of premature labor. On April 5 it was found that this pig had aborted during the night, giving premature birth to three fetuses, weighing altogether 120 gms. These were covered with hair and measured 10.5, 10, and 9.5 cm. in length. This pig remained well during the next few days. On April 9 she weighed 730 gms. and was returned to piggery.

EXPERIMENT 5. *Magnesium lactate*.—A sterile solution of magnesium lactate was prepared containing 2.3017 gms. $\text{Mg}(\text{C}_3\text{H}_5\text{O}_3)_2$ in 100 c.c., by neutralizing 21.1 of N/1 lactic acid with pure magnesium oxide. A healthy female guinea-pig, weighing 900 gms., in the fifth to seventh week of pregnancy, received by intraperitoneal injection 9 c.c. of the above solution of magnesium lactate at 12:30 P.M., on April 1, 1912. She showed no discomfort from the injection, but during a short interval after the injection she manifested a desire to lie down and seemed in a somewhat relaxed condition. During the remainder of the afternoon she remained normal. On the morning of April 2 the pig seemed well but had a marked diarrhea. There was no evidence of premature labor. On April 3 the pig had recovered from the diarrhea. There was no evidence of premature labor. On April 4 the pig seemed well—still no evidence of premature labor. On April 5 she seemed well and weighed 780 gms. No interference with pregnancy. On April 8 she weighed 740 gms. and on this date received by intraperitoneal injection 9 c.c. of a mixture of 25 c.c. of normal colostrum of the cow and 5 c.c. of distilled water. The liquid injected was previously heated to 38°. The injection caused some discomfort. On April 9 the pig seemed well and had not aborted. On April 10 she seemed well, weighed 680 gms., and had not aborted. On April 12 she seemed well and had not aborted.

It is evident from these results, therefore, that under certain conditions at least, and so far as we have been able to ascertain under normal conditions of pregnancy, calcium salts in the amounts and at the concentrations indicated above are specific in giving rise to premature onset of labor in pregnant guinea-pigs, within a very short interval following their administration intraperitoneally. In the light of Bell and Hick's observations they do this by causing a rhythmical, expulsive contraction of the pregnant uterus. The fact also that potassium lactate, though greatly slower in its action, can accomplish the same thing is also a matter of interest. That sodium lactate, in the amount and at the concentration here employed, does not bring on the onset of premature labor in the pregnant guinea-pig, is what one might naturally be led to expect from the general inertness of small amounts of sodium salts in the

animal organism. The fact also that magnesium lactate, in the amount and at the concentration here employed, is powerless to bring on the onset of labor is also what we would be led to anticipate from the work of Meltzer and Auer¹ on the physiological action of magnesium, which, in the form of its soluble salts, greatly inhibits many vital processes and causes complete relaxation, general anaesthesia, and ultimately death. Our guinea-pig gave some evidence of a slightly relaxed condition immediately following the intraperitoneal injection of magnesium lactate; and in this connection it is interesting to note that thus far fresh, normal colostrum from a healthy cow has failed to bring on the onset of labor in the pig that received the magnesium lactate, indicating possibly a protective action on the part of the magnesium against the calcium salts of the colostrum. We hope to continue these investigations.

¹ *Am. Jour. Physiol.*, 1908, 21, p. 400.

ON THE DEVELOPMENT OF PROTEOLYTIC FERMENTS IN THE BLOOD DURING PNEUMONIA.*

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In the pneumonic crisis we have an extremely rapid recovery which, could it be brought about at will, would represent an ideal therapeutic result. An understanding of the processes responsible for this change is therefore especially desirable.

With the hope of finding differences in the blood of pneumonic patients before and after crisis, which might be held accountable for its occurrence, the various antibodies commonly developed in infectious diseases have been extensively studied. Recently the opsonic and phagocytic power of the blood has received the greatest amount of attention (Wolf, MacDonald, DeMarchis). Rosenow¹ also studied the opsonic power of the serum of pneumonic cases, using non-virulent strains, and found a constant increase in opsonic content of the blood at the time of crisis. With virulent organisms, however, phagocytosis did not occur. Later, in a study of the lung exudates obtained by aspiration from the lungs of patients,² he found that phagocytosis occurred, but that whenever it was found the organisms outside the leukocytes showed evidence of disintegration. Rosenow concludes that while the opsonins take part in the defense of the body against pneumococci, their rôle in the process of the crisis is a secondary one. Strouse³ reaches a similar conclusion. Eggers⁴ obtained by the plate method evidences of an increased antipneumococcal power of the blood at or shortly after the crisis, and attributes this action, at least in part, to opsonins. Neufeld and Händel⁵ claim that the serum of postcritical pneumonia patients protects mice against fatal doses of pneumococci. Neufeld and Händel⁶ and Kyes⁷ have produced serum by immunization which protects mice against pneumococcus infections. Moro⁸ observed an increase in the complement content of the blood after crisis.

The importance of the splitting of bacterial proteins in infections has been established by Vaughan,⁹ Friedberger and his associates,¹⁰ Pfeiffer and Mita,¹¹ Rosenow,¹² and Neufeld and Dold.¹³ These investigators have shown that increased toxicity occurs with proteolysis up to a certain point beyond which further splitting decreases the toxicity of the protein. The production of protein-splitting ferments by the injection of protein antigens has been demonstrated by Abderhalden and his

* Received for publication April 17, 1912.

¹ *Jour. Infect. Dis.*, 1906, 3, p. 683.

⁴ *Jour. Infect. Dis.*, 1912, 10, p. 48.

² *Ibid.*, 1911, 8, p. 500.

⁵ *Arch. a.d.k. Gsndhtamte.*, 1910, 34, p. 166.

³ *Jour. Exper. Med.*, 1911, 14, p. 109.

⁶ *Loc. cit.* ⁷ *Jour. Am. M. Ass.*, 1911, 56, p. 1878.

⁸ *Ueber das Verhalten hemolytischer Serumsstoffe beim gesunden und kranken Kind*, Wiesbaden, 1908.

⁹ *Jour. Infect. Dis.*, 1907, 4, p. 476.

¹⁰ *Deutsche med. Wchnschr.*, 1911, 37, p. 377.

¹² *Jour. Infect. Dis.*, 1912, 10, p. 113.

¹¹ *Ztschr. f. Immunitätsf.*, 1911, 6, p. 18.

¹³ *Berl. klin. Wchnschr.*, 1911, 48, p. 1069.

associates.¹ I have shown² that these proteolytic ferments may be specific and capable of inactivation and reactivation.

The following experiments were attempted in order to find out if such ferments were demonstrable in pneumonia or not. On account of the importance of complement in anaphylaxis³ the complement estimations of Moro were repeated.

An extract of pneumococci was prepared by growing virulent pneumococci in ascites or serum broth in large quantities and centrifugating the cultures. The broth was then pipetted off and the bacterial sediment heated to 60° C. for one-half hour in order to destroy the autolytic ferments. The heated bacteria were then dried and ground thoroughly with sand and extracted with salt solution for 24 hours in the ice-box. The salt solution used was half normal so that the strength of the extract could be varied by adding salt solution or by evaporation. The extract was then cleared by centrifugation, filtration through paper pulp, and finally when necessary through porcelain filters. The extract was now diluted so as to give a levorotation of about 30'-45' with a 10 c.c. tube, and then sterilized by heating to 60° C. for one hour.

Blood was obtained from the arm veins of pneumonia patients at various periods of the disease, before and after crisis, and the serum removed by centrifugation. A mixture of 1 c.c. of serum and 10 c.c. of pneumococcus extract was made and the optical activity estimated as quickly as possible after mixing thoroughly. The mixture was then incubated for four hours and a second reading made. The results are given in tabular form.

The complement was estimated by the following method: Tubes were arranged with 0.1, 0.2, 0.3, 0.4, and 0.5 c.c. of a dilution of 1-100 of the serum to be tested. A corresponding set made with normal serum served as a standard. To each tube was added 10 times the minimum amount of amboceptor required to produce complete hemolysis with 0.1 c.c. of a 1-10 dilution of normal serum; 0.2 c.c. of a 5 per cent suspension of sheep's blood were then added and the mixture made up to 1 c.c. Controls were made without amboceptor to guard against the estimation of the

¹ *Ztschr. f. physiol. Chem.*, 1910, 64, pp. 100, 423, 425, 427.

² *Jour. Infect. Dis.*, 1911, 9, p. 282.

³ Friedberger and Hartoch, *Ztschr. f. Immunitätsf.*, 1909, 3, p. 581.

total hemolytic power of the serum rather than the complement concentration. The mixtures were incubated for two hours and then placed on ice for 18-24 hours and examined. The figures in the table refer to the number of 0.1 c.c. parts in the test serum and the standard serum giving the same amount of color to the fluid over the sedimented corpuscles; for instance, 3 would mean that 0.1 c.c. of the test serum equaled in complement action 0.3 c.c. of normal serum, $\frac{1}{3}$ would mean that 0.3 c.c. of test serum equaled 1 c.c. of normal serum.

CASE	DESCRIPTION OF CASE	OPTICAL ROTATION OF MIXTURE IN MINUTES		DIFFERENCE IN ROTATION	COMPLEMENT
		Before Incubation	After Incubation		
1	Third day of disease	40'	40'	0'	1
2	Fourth day of disease	48'	48' ?	0'	1
3	Sixth day of disease	37'	37'	0'	1
4	Eighth day of disease beginning lysis	47'	46'	1'	$\frac{1}{2}$
5	Normal serum	41'	40'	1'	1
6	Normal serum	37'	36'	1'	1
7	Two days after crisis	39'	31'	8'	$\frac{3}{4}$
8	Three days after crisis	46'	40'	6'	1
9	Four days after crisis	42'	35'	7'	2
10	Six days after crisis	39'	35'	4'	2
11	Ten days after crisis	43'	34'	9'	$1\frac{1}{2}$
12	Ten days after crisis	40'	33'	6'	2
	Extract alone, control	31'	30'	1'	
	Serum from case 9 alone	12'	12'	0'	

The table shows a distinct decrease in the optical activity of the mixtures made with the serum after crisis and none with the normal serum or the precritical pneumonic serum. Case 10 represents an exception to this as well as one other case (14). In neither case was there anything peculiar in the clinical course to explain the difference. Inasmuch as blood cultures were not made, however, it is possible that these were not pneumococcus infections, although they represented clinically typical cases. According to Abderhalden, this decreased optical activity would indicate that digestion of the extract had taken place. In order to decide whether the ferments in postcritical serum were specific or whether they might be connected with the process of the resolution and removal of the pneumonic exudate, serum from postcritical pneumonics was compared as to its action on pneumococcus extracts, colon bacillus extracts, and typhoid bacillus extracts. The results are as follows:

Case 13, Comp. = 1	Pneumococcus Extract	Colon Bacillus Extract
Before incubation.....	54'	48'
After incubation.....	50'	47'
Difference in rotation.....	4'	1'

Case 14, Comp. = 1	Pneumococcus Extract	Colon Bacillus Extract
Before incubation.....	47'	40'
After incubation.....	45'	40'
Difference in rotation.....	2'	0'

Case 15, Comp. = 2	Pneumococcus Extract	Typhoid Bacillus Extract
Before incubation.....	48'	49'
After incubation.....	44'	50'
Difference in rotation.....	4'	1'

These results would indicate that the proteolytic action was specific for pneumococci.

It will be noted that except in cases 8 and 13 the hemolytic complement content of the blood is higher in those cases having a proteolytic power. In order to further investigate this relationship the serum of case 9 (after crisis) was heated to 60° C. for one-half hour and a mixture made of 0.5 c.c. of heated serum, 1 c.c. of normal serum, and 10 c.c. of pneumococcus extract. The polariscopic reading was 48', and after incubation 44'. Two serums having different proteolytic power (cases 1 and 7) were heated to 60° C. for one-half hour and then mixtures made of 1 c.c. of heated serum, 0.5 c.c. of guinea-pig serum, and 10 c.c. of pneumococcus extract. The results were as follows:

Case 1.	Before incubation	41'	After incubation	40'
Case 7.	"	"	"	38'
		41'		

It will be seen that although the guinea-pig serum contains about eight times the hemolytic complement content of normal human serum and that although the content complement in guinea-pig complement was made equal in the serums of cases 1 (before crisis) and 7 (after crisis), there is still a difference in the proteolytic action. The significance of the difference in hemolytic comple-

ment content of the blood before and after crisis requires further investigation.

CONCLUSIONS.

Proteolytic ferments develop in the blood during pneumonia about the time of crisis. These ferments seem to have a special action upon pneumococcus protein and may take part in the mechanism of the crisis.

AN OUTBREAK OF TYPHOID FEVER IN CEDAR FALLS, IOWA.*

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Cedar Falls is a city of about 5,000 inhabitants, situated on Cedar River about seven miles above Waterloo in Blackhawk County. The Iowa State Teachers College is located in the city and has an attendance of about 1,100. As many of the students are regular residents of Cedar Falls the combined population is in the neighborhood of 6,000. The city is principally a residential one, being the home of well-to-do people, many of whom are retired farmers and their families. There is a flour mill and several smaller manufacturing establishments in the town but none of these employ very many hands. Outside of the students, who are somewhat overcrowded in rooming- and boarding-houses, most of the people live in their own homes—that is, are not lodgers.

About November 1, 1911, several cases of typhoid fever developed in the city. At the request of Dr. Albert, and by permission of John Bowman, president of the university, I was assigned to represent the State Board of Health in order to make an epidemiological investigation of conditions at Cedar Falls. This investigation was started November 9, 1911. Attempts were at once made to prevent further infection, by publishing in the local papers a notice which was signed by the mayor of the city (in Iowa the mayor is president of the Board of Health, the city council being the board), warning the public against uncooked foods.

Samples of water were taken at various points from the public supply and sent to the laboratory for bacteriological examination. The study of each individual case was now commenced by visiting the homes of those reported sick with typhoid fever. As the disease is not a reportable one in Iowa, although Cedar Falls had an unenforced ordinance compelling the report of same, I was obliged

* Received for publication April 9, 1912.

to obtain my reports of cases from the local physicians. In every instance I found the physicians willing and even anxious to facilitate the work. For obtaining the necessary data a very full inquiry blank was filled out in each case.

Each night on returning to the hotel the data obtained in this way during the day were tabulated, and from the resulting information my attention would be directed to whatever phase of the subject might be brought to the front. After 80 cases had been thus thoroughly investigated it seemed as if we had enough knowledge on which to make final conclusions. These facts and conclusions will be briefly enumerated so that the results and the manner in which they were obtained may be seen.

Four cases of typhoid fever occurred during July, 1911, two of which were apparently due to swimming in the river and the other two were traced to carriers. These appeared to have nothing to do with this epidemic. Of the 80 cases of typhoid fever investigated all were white, 34 were male, and 46 female.

As compared with previous epidemics we had the following:

PERCENTAGE OF TYPHOID FEVER AT SPECIFIED AGES DURING VARIOUS OUTBREAKS.

AGE IN YEARS	WATERVILLE, MAINE.* 1903 CAUSED BY WATER	STAMFORD, CONN.* CAUSED BY MILK	DES MOINES, IA.* 1910 CAUSED BY WATER		TEXARKANA, ARK.-TEX.† CAUSED BY MILK	CEDAR FALLS, IA.‡ 1911
			Investigator		Investigator	Investigator
			Sayles	Lumsden	Redlon	Grover
0-10.....	17	35	14	20	40	15
10-20.....	38	24	43	44	42	38
20-30.....	26	23	26	28	12	32
30-40.....	10	12	11	4	6	14
40-50.....	4	5	5	4	0	3
50-70.....	5	1	1	0	0	1
Totals...	100	100	100	100	100	100

* *Public Health Rep.*, U.S. Public Health and Marine Hospital Service, 1911, 26.

† *Ibid.*, 1912, 27.

‡ *Rep. to William H. Merner, Mayor of Cedar Falls*, Nov. 20, 1911.

The percentages in this outbreak, given in the above table, resembled more closely the ones obtained from outbreaks that were due to water rather than those due to milk. Further comparisons might be made but seem unnecessary.

The accompanying charts showed the dates of the onset of first symptoms of those infected and the dates when the patients first

took to their beds. The four cases in July and August have been omitted.

From these we saw that the beginning of the epidemic was about November 4, that November 8 was the high point, and that from then on it gradually diminished. The difference in the dates of

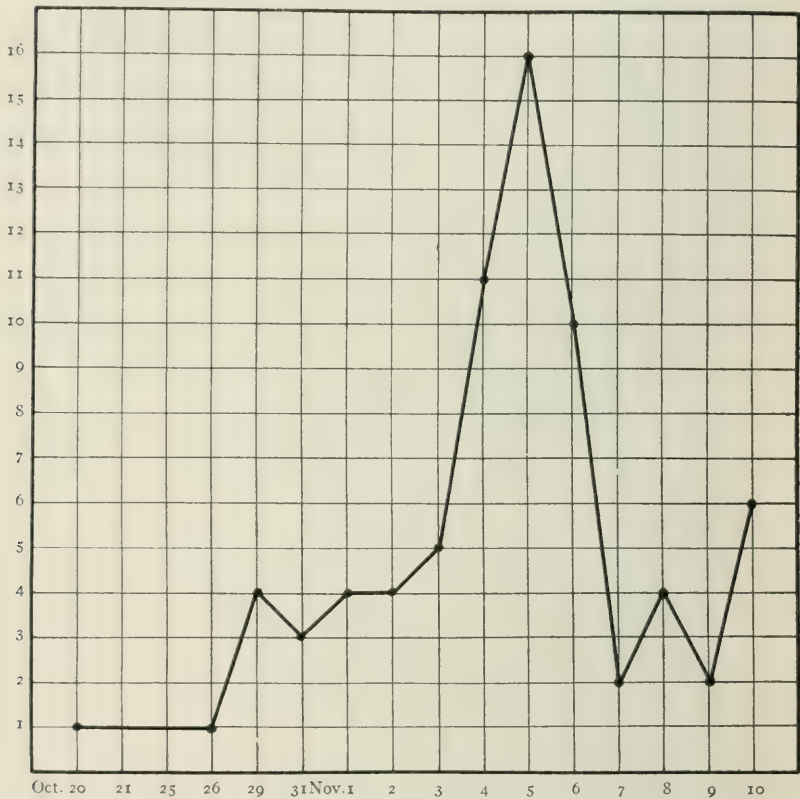


CHART 1.—Showing date when patients first felt ill.

first symptoms and those of first going to bed gave marked evidence of prodromal symptoms. We allowed 14 days as the incubation period for typhoid fever and counted back from November 5. This gave October 21 as the approximate time of infection.

At the time of my investigation there were reported to me by the physicians 95 cases of typhoid fever, 80 of which were examined. Since that time there have been reported to the mayor, including

the above, about 170 cases. These, with reports from other parts of the state of persons ill with the disease who undoubtedly contracted it in Cedar Falls, make a total of at least 200 infections. The death-rate has been about 10 per cent, which compares favor-



CHART 2.—Showing date when patients first went to bed.

ably with the usual water-borne typhoid fever epidemic death-rate. The diagnosis was confirmed in a great many of the cases by the Widal test. As in all epidemics, there were some cases reported as typhoid that could not be proved, and on the other hand there were, as usual, many more cases that were light and passed

unnoticed. The students of the Teachers College went home in numbers and carried the disease to many parts of the state.

Nearly all of the persons infected, except the students, had lived in their residence for more than a year—the students about 10 weeks. Thus previous residence was excluded as having any bearing on the outbreak. The occupations had nothing to do with the infection, as is shown by the following table.

OCCUPATIONS OF THOSE INFECTED WITH TYPHOID FEVER.

Infant	2	Driver	1
Retired	1	Servant	1
Student (college)	18	Electrician	1
School child	30	Farmer	1
Housewife	8	Post-office clerk	1
Bookkeeper	2	Conductor	1
Undertaker	1	Librarian	1
Teacher (public school)	2	Grocer	1
Teacher (college)	1	Not obtained	3
Laborer	4		
			80

There was a greater proportion of the cases among scholars and students, but these of course were all at the typical "typhoid age." The table of ages has already shown that there was no great proportion of very young children infected. Then again, nearly all the children of school age in this community are to be found in school rather than at work, as in many other places.

The places of business of those infected were distributed well over the city and the school children evenly distributed throughout the schools in proportion to each school's attendance. The sanitary conditions of the premises were excellent. Sixty of the houses were connected with the public sewer. The privies in some of the other places were open to objection on account of faulty construction and improper care, but these very evidently had no connection with the source of infection. There were a few cases where old wells driven down to the limestone foundation were used as cess-pools—which seemed a most dangerous and pernicious practice and one that should be absolutely forbidden.

The season, the distribution, and the general character of the outbreak entirely eliminated flies and other insects as the cause of the outbreak. Every house had been screened during the previous summer. There had been no unusual amount of wind and

apparently no dust at time of infection. Also there was no history to be obtained that would point to either carriers or contact as a cause of the epidemic. From the explosive type of the outbreak we know that the infecting agent must have been spread by some beverage or food. Outside of the students nearly all obtained their food at home. As to the student boarding-houses, the cases were scattered pretty well throughout all, and no connection was to be found as to carriers, etc.

The milk supply was most carefully examined, both from the inquiry into the source of supply of those sick and from a most careful sanitary inspection of the dairies involved. Forty of the cases had taken milk as a beverage, 20 on cereals only, three in tea or coffee only, 13 denied using milk in any form, and information could not be obtained in one case. Thus we see that 16 per cent of those sick with typhoid fever used no milk at all. The milk dealer with the greatest number of customers had the most cases among his patrons—in other words, no one milk dealer had a number of cases disproportionate to the size of his business. The following table shows the number of cases taking milk from each distributor.

Milkman "A".....	21	Milkman "K".....	1
" "B".....	10	" "L".....	1
" "C".....	4	" "M".....	1
" "D".....	4	" "N".....	1
" "E".....	2	" "O".....	1
" "F".....	2	" "P".....	1
" "G".....	2	" "Q".....	1
" "H".....	2	Have their own cow.....	6
" "I".....	2	No milk used.....	13
" "J".....	1	Information not obtained.....	4
			80

In epidemics that have been traced to milk as the inciting factor we have generally seen that there were apt to be several cases in one family, that prodromes were absent, due to virulence of the infecting organisms—milk being such a good culture medium for the typhoid bacillus that it would be present in immense numbers—and that those of the so-called "milk-drinking age" (young children) were more apt to be infected. Those of any age drinking milk as a beverage of course will show the greater proportion of

infections in such an epidemic. This condition was not manifested in Cedar Falls.

There were many cross clues to work out on the milk question and it took some time and an immense amount of work successfully to rule out milk, although in the main the evidence tended from the start to point away from milk as the incitor of the outbreak. It was found, as stated above, that the milk drinkers were not especially affected, nor the young (of the milk-drinking age), nor were prodromes absent, nor were there more than one case per family except in a few instances. Many dairies were visited and samples of their well-water taken and examined at the laboratory. These sanitary examinations showed absolutely no evidence of carriers or contaminated wells where the milk was produced. One of the first cases in July, which I have already referred to as probably having been infected from swimming in the river, was in the milk business until the day before he died. While sick he apparently had very little care and was accustomed to sleep on his ice-chest in the cellar in order to try and keep cool. He was evidently up and about more or less all the time. Why an epidemic did not start then is hard to say. The day previous to his death he sold out to another man. This second milkman in October also sold out his business to milkman "A" and went to work for milkman "B," delivering but not otherwise handling the milk. He soon developed typhoid fever and died. The last two weeks that he worked he undoubtedly had the disease. His death took place around the first of November. Milkmen "A" and "B" had together 31 of the cases, or 38.75 per cent of the total. Hence it will be seen that this all taken together rather complicated the situation. However, everything taken as a whole, when all the dairies had been investigated and all other data obtained, pointed against milk being the infective agent.

Forty-one of those sick with typhoid fever had eaten ice-cream, of whom 16 obtained it at one place, eight from another place, and the other 17 at both places or at home. Both ice-cream manufacturers had their own private source of cream and denied any trading from one to the other—in fact they appeared rivals rather than friends. The first place had twice during October bought

cream of milkman "A." This further complicated matters. Further investigation showed, however, that none of this cream was made into ice-cream but was all retailed over the counter. All the dairies supplying these places were inspected but no possible source of infection was found. Milk and ice-cream were finally ruled out as source of infection from the fact that the disease as manifested did not resemble a "milk type" of infection and for the other reason given above.

All the grocery stores in the city, with one exception, got their butter from a local creamery which collected cream from 250 farms and dairies in the surrounding country. The owners of this creamery appeared to be keeping a very careful watch over their producers and had reports of sickness as to only two places (one scarlet fever and one small-pox), the milk from which had been immediately discontinued. It was of course impossible to look into all these sources of cream. Also, two of the cases of typhoid fever bought their butter from the store not supplied from this creamery and it was learned that many of the college boarding-houses obtained their supply of butter from farmers who brought it in from the surrounding country. On these grounds further consideration of butter was given up.

Apples and grapes had been eaten by about all of those sick with typhoid fever, but the sources of supply were so various and scattered that it would have been unreasonable even to have considered them. Celery had been eaten by less than 50 per cent of those infected. Many of the people raised their own celery and the remainder obtained that which had been about all raised by one gardener who used deep well-water with which to irrigate his land and wash the celery. He used no "night-soil" as fertilizer. The water from the well on examination proved to be good. Therefore, on account of the rather small percentage of cases who had used this celery and from other data obtained in regard to other phases of the subject, celery was ruled out as the inciting factor. Other vegetables were eaten raw but by a very small percentage—less than 25—and the vegetables and their source were, as in the case of apples, varied and scattered. Raw shellfish had been eaten by only one or two. Only a few persons had taken a trip out of

town at the time of infection. There was no history of previous typhoid or intestinal trouble to be obtained, either in the household where sickness was found or in the households of their servants. In this manner carriers as the source of infection were ruled out.

The city water was the source of supply for all the residents of Cedar Falls who contracted typhoid fever in this outbreak, except in two cases—one of which worked where he drank city water and the other attended public school where city water was furnished. Three cases outside the city limits had their own private water supply—but they all obtained city water either at their places of business or on daily trips to town.

The city of Cedar Falls had always prided itself on the purity of its water supply. Chemical analysis had failed to detect any pollution with sewage material. The chlorine and nitrate content were always high and showed some variation, but such had been explained as coming from natural sources. According to Dole,¹ this variation pointed rather strongly to surface water pollution. The source of water supply is a collection of springs at the foot of a hill southeast of the city, along the banks of a "dry run" which empties into the Cedar River about 200 yards below, after passing under the tracks of the Chicago, Rock Island & Pacific Railroad. The sanitary sewer of the city empties into the river a short distance above this point. The location of these springs is lower than the city itself and also lower than most of the surrounding country. The water-shed comprises about 30 square miles. These springs cannot be seen, as they are inclosed with brick and roofed over with some sort of material. Other springs (one of which is known as the Pfeiffer) are to be seen gushing forth large streams below, along the banks of the "run." From the inclosure noted above, an overflow pipe of iron, fitted at the end with a swinging trap valve, empties into "dry run." A wooden conduit (iron under "dry run") conducts the water by gravity from the spring about 1,000 feet northeast to a brick and cement collecting cistern from which it is pumped directly into the city mains. The pumping station is situated beside the cistern. The excess pumpage over-

¹ *Rep. to Director U.S. Geological Survey, 1911; Cedar Falls Daily Record, January 15, 1912.*

flows from the mains into a water tower some distance from the water plant on a high ridge of land in the central portion of the city.

The formation from which these springs issue is what is known as the Cedar Valley Limestone and is a broken formation full of faults and water runs. Most of the drilled wells in the surrounding country go down to this same stratum. It outcrops in the bed of this "dry run" and can be seen in several quarries upstream along its course. This "dry run" some distance from the springs disappears in its gravelly bed and possibly reappears again below. It is admitted by all that during high water or after severe rains the water from these springs is apt to be roily; also that the water in both the drilled wells and the quarries mentioned above becomes roily. These facts, together with the fact that the geological formation tends to intercommunication between these various sources of ground water, show that at times there certainly has been contamination with surface water. Whether this contamination comes from the overflowing river or from the water-shed itself cannot be ascertained. We do know that crevices into which brooklets run and disappear are present along "dry run." These crevices have in the past been sealed up whenever found. We know also that the overflow pipe from the spring is under water when the river is high enough to back up along "dry run"; that the collecting cistern has no impervious bottom; and that the wooden conduit is not water tight. These last two portions of the system were constructed under water, owing to the springy condition of the ground. It would seem therefore that there was and is yet a chance for contamination at all these points.

On October 19 and 20,¹ the flour mills in Cedar Falls had to shut down on account of high water, the rise being of about four and a half feet and taking several days to return to normal. It requires about a two-foot rise to cover the spring overflow. This high water followed the flooding of the headwaters of the river and did not take place until four or five days after the heavy local rains in October. Now the previous season was one of almost no rainfall

¹ According to the records of Superintendent John Lemmer of the Waterloo and Cedar Falls Union Mill Co.

and this October rain had therefore an unusual amount of surface contamination to wash into the river. The river thus became a much more concentrated source of contamination than for a long time previous. The time of the high water and that of the infection were the same. On October 20 or 21 the college filled its swimming pool and made a somewhat extra draw in that direction. It was noticeable that a large number of cases appeared in the general direction of the college and along the principal main. The standpipe takes the excess pumpage by a pipe running to it from the principal main. It is possible that infected water may have been pumped into the tank at night when little was being used and distributed the following morning. Water has been seen to be the only thing that was common to all. It could easily have been contaminated from the river, from crevices along "dry run," from seepage into wooden conduit, into the cistern, or into spring itself. Bacteriological examination of the water at different times varies considerably and shows some possibility of contamination. It certainly shows more variation than a deep ground water should. The following table gives these analyses previous to reporting. Analyses made since show no evidence whatever of contamination.

SAMPLE No.	SOURCE	COLONIES			GAS IN	
		At Room Temp.	At Incu- bator Temp.	Of Colon Group	1 c.c.	2 c.c.
1513 . .	City water not packed properly (not examined)					
1515 . .	City water	30	4	3	None	None
1522 . . .	Pumping station (not iced)	140	35	5	95 per cent	20 per cent
1523 . .	Pumping station (iced)	7	3	0	Trace	Trace
1526 . . .	Spring overflow (iced)	480	40	0	30 per cent	60 per cent
1535 . .	Pumping station (iced)	4	3	0	60 per cent	Trace
1539 . .	Pumping station (iced)	3	12	0	Trace	Trace
1537 . . .	Spring overflow (iced)	12	1	0	"	30 per cent
1539 . .	City water (iced)	4	1	0	"	Large trace
	Burr House					
1541 . .	City water (iced)	20	3	0	"	" "
	Burr House					
1527 . .	Pool near pumping station (condensation water from pumps)	2,000	1,500	0	55 per cent	48 per cent

The following were the conclusions given as to the source of the epidemic:

"I feel convinced that the city water was the source of the epidemic for the following reasons:

- "1. Every patient used the city water.
- "2. Every other cause of infection can be absolutely eliminated.
- "3. Laboratory examinations show at times pollution of water with sewage material.
- "4. City water becomes cloudy after high water, indicating that it is subject to change in the surface soil.
- "5. Time of infection corresponds with high-water period.
- "6. The construction of the water plant is such as to permit of pollution.
- "7. The emptying of the standpipe may have influenced the determination of water polluted at that particular time in the direction of the standpipe and account for the great number of cases along main leading directly into or out of it.
- "8. The season of occurrence, the extent and the explosive character of the outbreak are all in favor of water being the cause of the epidemic."

During the investigation it was advised that the water be treated with hypochlorite of lime. This was at once done. In the final report made it was also recommended:

- "1. That there be instituted a system of public water supply which shall be free from the possibility of pollution with sewage material.
- "2. That the ordinance relating to the reporting of cases of typhoid fever be very strictly complied with.
- "3. That the city sewerage system be extended as rapidly as possible to all parts of the city.
- "4. That privy vaults be abolished in all places where it is possible to connect with the sewer or where such connections have already been established.
- "5. That all drilled wells used as cess-pools be abolished."

One recommendation made, viz., that the people become vaccinated against typhoid fever, was readily taken up. The following data as to the results of this vaccination were obtained from local physicians.

1. Number of persons vaccinated against typhoid fever between January 1, 1911, and February 1, 1912, 911.

2. Number of persons vaccinated who contracted typhoid fever, 12.

Number after one inoculation, 4.

How long after

{ 7 days
6 weeks
8 days
8 days

Severity of disease, all mild.

* *Rep. to William H. Merner, Mayor of Cedar Falls, November 20, 1911.*

Number after two inoculations, 3.	How long after	$\left\{ \begin{array}{l} 4 \text{ days} \\ 3 \text{ days} \\ 7 \text{ days} \end{array} \right.$
Severity of disease		$\left\{ \begin{array}{l} 2 \text{ died} \\ 1 \text{ mild} \end{array} \right.$
Number after three inoculations, 5.	How long after	$\left\{ \begin{array}{l} 3 \text{ days} \\ 2 \text{ years} \\ 2 \text{ weeks} \end{array} \right.$
Severity of disease, all mild.		$\left\{ \begin{array}{l} 2 \text{ weeks} \\ 2 \text{ weeks} \end{array} \right.$
Number after four inoculations, 0.	How long after	—
Severity of disease —		

Number of persons vaccinated who were nursing cases of typhoid, 88.

Number of these who contracted typhoid, 0.

Number not vaccinated and nursing cases of typhoid, who contracted the disease, 12.

We find that 911 persons were vaccinated at this time and one two years previous, which, with 12 infections, gives a percentage of infection of 1.2. The population of Cedar Falls plus the students is about 6,000. Estimating the percentage on a basis of 170 cases, we have a percentage of infection of the whole population of 2.8. It is almost impossible to figure percentages in this epidemic, since we have no exact knowledge of the time when the last 90 cases took sick. Everyone probably was exposed to the infection at the same time. Over half of the cases were taken sick before vaccination was begun, so that the percentage of cases developing afterward was somewhat reduced. None of the first hundred cases had been vaccinated. The percentage of infection in those vaccinated was, as stated above, 1.2, which, compared with 2.8 per cent in the case of the total population, seems to indicate that vaccination will at least reduce the number of secondary infections. It also seems to have a tendency to reduce the severity of the infection, for nearly all those given in the table above were infected before vaccination was begun. The death-rate of the whole epidemic was about 10 per cent, of those infected after vaccination, 16.6 per cent. Both of the deaths were in cases with a particularly virulent type of the disease, which were infected before vaccination. The mild course of all the other cases would seem to indicate that as a general thing vaccination tends to reduce the severity of infection whether patient became infected before or after the vaccina-

tion. This is borne out by the fact that after three inoculations there were no deaths and a mild infection only. The fact that 88 nurses were vaccinated with no infections is certainly significant when compared with the fact that there were 12 infections in the non-vaccinated nurses. The number of nurses is not known, but allowing one for a case, we would have an infection percentage rate in the 88 nurses not vaccinated of 14.6.

Although the epidemic was disproportionately large for the size of the city, the conditions were handled in the best possible way. Nearly every patient had a trained nurse—the state maintained two hospitals for the students and the city one for those without homes. Every precaution was taken, which together with the vaccinations did much to prevent secondary infections.

As a result of these epidemiological findings Professor A. Marston from an engineering standpoint made the following recommendations:¹

"1. I recommend that the city of Cedar Falls at once instal permanent reliable apparatus for sterilizing the present spring water supply with hypochlorite of lime, and that the city continue such sterilization with all possible precautions, *at all times when the spring water supply is used.*

"2. I further recommend that the city of Cedar Falls proceed at once to sink an artesian well similar to those at Waterloo, and to properly equip it with pumping apparatus, pump house, and clear water basin, all at an estimated total cost of \$15,000. I recommend that when artesian supply is developed the present spring water supply be used only as a reserve in case of breakdown and only with proper sterilization.

"3. I further recommend that in due time a second artesian well be sunk, and the spring water supply abandoned entirely."

For safety while the wells are being installed he also advised the construction of a sort of chimney on roof of spring inclosure with a manhole at top above high-water level so that the condition of the spring water might be determined at high water. This has been done.

On December 30, 1911, R. B. Dole, chemist of the United States Geological Survey, was sent to Cedar Falls to make a sanitary survey of the water supply—necessarily limiting his inquiry to what would be pertinent to the Survey's investigation of Pollution of Rivers of Iowa, that is, to sources of ground water pollution. At his request I was present and assisted him in this phase of the

¹ *Rep. to C. H. Streeter, Superintendent of Water Works; Cedar Falls Daily Record, January 8, 1912.*

investigation from a bacteriological standpoint only. His description of the water plant and supply is in much more detail but yet essentially the same as I have given. He made a most exhaustive geological survey and found the condition of the Cedar Vally Limestone as has been explained. An attempt was made on the so-called "Carpenter quarry" in a crevice, and in the bed of "dry run" itself at varying distances above the spring, to prove that contamination with surface water could take place, by using fluorescein. Owing to the amount of snow on the ground and the severe cold freezing the ground, or because the right place was not selected, no results were obtained. There did not seem to be enough water present at the time properly to wash the coloring matter into the ground. Possibly this will take place with the spring thaws.

Mr. Dole gives the following comparative table of chemical analyses:

(Parts per Million.)

Date	Analyst	Fixed Solids	Total Solids	Chlorine
8- 8-'05	Bates	288	432	7.0
9-26-'05	"	250	382	6.75
2-26-'07	"	190	242	3.0
11- 3-'08	Kinney	234	342	5.5
12- 5-'09	"	144	201	4.75
12-12-'10	"	106	306	5.76
11- 7-'11	" *	104	324	7.12
11- 7-'11	" *	155	311	7.0
11- 7-'11	" *	102	413†	7.25
11- 7-'11	" *	109	310	6.50

* Samples from four different taps.

† Evidently an error in computation. Should be 313.

In his report he goes very carefully into the questions of filtration of the river water and of artesian wells. He appears to think it is a matter to be decided only after long and careful tests. He thinks the question of forming a water district with Waterloo should be looked into. He sums up as follows:

"As it is apparent that the water in the limestone immediately underlying the city is liable to dangerous contamination, measures should be taken to abandon this source of supply not only by the public but by individuals, and to further this action the use of water for domestic purposes from wells entering this limestone on the shallow beds of gravel overlying it should be prohibited by the city authorities. The best way to insure such abandonment is to remove the pump and fill up the well. The use of privies and cess-pools wherever connection can be made with the city sewerage should be prohibited, and every effort made to extend the sanitary sewerage through the city."

At about the same time Clark H. Streeter, city engineer and superintendent of water works, recommended that certain improvements in the line of safety be carried out while the question of obtaining a new supply was being considered. In the main these were: to replace the wooden conduit with a tight cast-iron gravity conduit to pumping station, to put a checking valve on spring overflow, to continue use of hypochlorite of lime, and to observe condition of spring at next overflow of river. He also recommended other improvements in the operating system.²

It is interesting to note that this community has had always present for years the danger of an epidemic of typhoid fever, yet the water only became contaminated for a short time and since has been as good as before. What is done to prevent future repetitions yet remains to be seen, but I feel that the community is one that will take the proper steps to safeguard its health.

SUMMARY.

The city of Cedar Falls, Ia., during November, 1911, suffered from an outbreak of typhoid fever. The time of infection was probably about October 20 or 21, 1911. The city water was used by all those afflicted, and, as all other possible causes can be ruled out, was undoubtedly the cause of the outbreak. There were about 200 cases with about 10 per cent of deaths.

Further investigations were made by Professor A. Marston and by Mr. R. B. Dole. Both agreed that the city water supply was the cause of the epidemic. All investigators, including Mr. C. H. Streeter, agreed on certain precautions to be taken immediately, and, in the main, on certain future improvements, particularly as to the fact that the city should seek a new water supply, although past and present analyses of the water show no particular pollution present.

The epidemic was handled in a proper manner and secondary cases well provided against by vaccination against typhoid fever, the results of this preventive treatment agreeing with previously published reports.

² M. F. Arey, professor of Natural Science in the Iowa State Teachers College, was able to render great assistance in pointing out the geological formations and establishing the drifts, dips, etc., of the water-shed.

COMPARATIVE TOXIN PRODUCTION IN DIPHTHERIA STRAINS.*

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The diphtheria antitoxin made by the New York Health Department is all produced by means of the action of a single strain of diphtheria organisms known in the laboratory as No. 8.

The organism used was obtained originally in the year 1895 in the course of ordinary routine culture examinations, from the throat of a child who had so slight an attack of diphtheria that it was at first diagnosed as tonsilitis. The animal tests which were being made with many strains at that time in the search for a good toxin producer showed this strain to be more active than any of those tried, and it was therefore selected for use in the preparation of toxin for the inoculation of horses. It has been constantly in use since that time for this purpose, has been kept growing in neutral broth, transferred about every third day, and has shown a remarkable persistence in vigor and toxin production. While this has fluctuated within wide limits at times, yet the careful studies made by Dr. A. W. Williams soon after isolation,[†] and confirmed by all subsequent work with the strain, show this fluctuation to be due to some alteration in the culture medium rather than to any inherent change in the organism itself, for a more careful preparation of the broth is always followed by a return to the same high toxin production, 1/400 to 1/500 c.c. and occasionally as high as 1/800 to 1/1,000, proving fatal within four days to medium sized guinea-pigs.

Morphologically the organism has remained unchanged during this long period of growth in the laboratory except that the rods are somewhat longer than when first isolated. In agar it has always produced very characteristic spreading colonies, and grows in broth with a typical heavy pellicle lying above a clear fluid.

Many comparative tests in the laboratory have always failed to find a culture stronger than No. 8 in toxin production, or even another equally active, but because of Dr. Park's desire to be thus

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† *Jour. Med. Res.*, 1902, 8, p. 83.

provided for the important work of toxin production it was decided to make another attempt, taking a large number of strains from different sources, and testing for toxin production soon after isolation, and again after a long period of cultivation; these results to be compared with those given by No. 8 grown under exactly similar conditions.

For this purpose 100 diphtheria cultures were obtained without selection, 25 being taken from the throats of diphtheria patients in the Willard Parker Hospital, and 75 from the cultures sent for diagnosis from the city to the Health Department laboratories. In the private patients, membrane was stated to be present on the throat in 67 cases, not mentioned in seven cases, absent in one case. Membrane was present on the throats of all the hospital cases, and generally in considerable amount, as nearly all were cases of a severe type. All strains without exception showed typical diphtheria bacilli, that is to say, forms corresponding to Wesbrooke's types A, C, and D. In most strains these were the predominating types, and in many almost the only forms present. Such strains gave from the beginning a vigorous growth in broth, with typical pellicle formation, and, on agar, characteristic spreading colonies. The other strains showed, besides the typical organisms, many smaller, more evenly staining forms on Loeffler's blood serum, and in most cases these strains grew with difficulty at first in broth, with pellicle either slight or absent, and on agar showed somewhat small dense colonies with but little tendency toward spreading.¹

All strains were carried on in neutral broth cultures at 37° C., transferred every third day, with frequent platings to insure purity. In those strains that were at first less characteristic, there was an increasing tendency in later cultures toward better growth, with pellicle production in broth and characteristic colony formation on agar, but in a few cases strains remained somewhat typical throughout in these media, although never failing to produce characteristic bacilli when grown on Loeffler's serum tubes.

After an interval long enough to allow the organisms to become accustomed to growth in an artificial medium, generally at about

¹ The individual organisms of these strains grown on agar were not studied, but they probably are strains producing large segmenting forms on agar as described by Williams.

the sixth to the 20th transfer, sometimes later, all strains were tested for toxin production.

Broth cultures grown in the incubator for eight days were examined by means of transfer to agar plates and blood-serum tubes, carbolic acid was added in 1 per cent amount, and after two days' interval subcutaneous inoculations were made into medium sized guinea-pigs. The dose ranged from 0.1 to 0.005 c.c., according to culture conditions and size of animals, while in a few of the less toxic strains as much as 3 c.c. was given in later tests. At the same time a corresponding test was made with the same quantity of a culture of No. 8 grown in the same broth, and under the same conditions.

The result showed that most of the strains, including some of those having the most characteristic cultural properties, produced much less toxin than the stock culture No. 8. Many failed to kill in less than 0.1 c.c. and a few were not fatal even in larger amounts; while death, when it did occur, was generally much later than in the control animals inoculated with No. 8. With a few strains in these early tests the results were at first more nearly parallel, guinea-pigs injected with 0.1 to 0.02 c.c. dying as soon as, and in one or two cases even a little earlier than, the corresponding control animal, but when successively smaller doses were given, in all cases there was a lengthening interval, the new strain always showing less toxicity than the stock culture.

All strains were now again carried on for some time in broth and after a varying number of transfers, generally from 30 to 40, and in those first isolated from 80 to 100, or more, the strains were again incubated for toxin production, together with No. 8, and tested as before.

The results were even more disappointing than in the earlier series as to finding a better culture than No. 8, or even a second equally active toxin producer. In some cases the amount of toxin production was comparatively unchanged, but the majority of the strains, among them some of those formerly giving the best results, now fell still farther behind No. 8.

The toxin from five strains failed to kill in less than 3 c.c. and that from nine others was not fatal even in this amount, but this result

was evidently largely due to the lack of ability in these organisms to grow well in the broth used, for when, at Dr. Williams' suggestion, they were cultivated for several transfers in ascitic growth and then tested for their virulence, in every case but one, death followed the inoculation of 0.1 c.c. of the living culture. This one was classed with the non-virulent and hence non-toxin producing organisms. The amount of toxin production in ascitic broth was not tested. Three other strains grew with such difficulty in ordinary broth that no satisfactory toxin tests could be made with the cultures, but grown in ascitic broth and tested for virulence, these strains also proved fully active in doses of 0.1 c.c. These results correspond exactly with those obtained by Dr. Williams in 1902. The best results in toxin production obtained with the entire series may be summarized as follows:

TABLE 1.

3 strains killed in 0.005 c.c.

11	"	"	"	0.01	"
25	"	"	"	0.02	"
6	"	"	"	0.033	"
11	"	"	"	0.05	"
27	"	"	"	0.1	"

The following table shows the relation between toxin production and virulence in the 17 strains producing little or no toxin in ordinary broth, and tested for virulence in ascitic broth.

TABLE 2.

TOXIN VIRULENCE.

NEUTRAL BROTH			ASCITIC BROTH	
Strain	Dose	Result	Dose	Result
8	3 c.c.	Lived	0.1 c.c.	x 3 days
31	3 c.c.	x 2 days	0.1 c.c.	x 2 days
32	3 c.c.	Lived	0.1 c.c.	Lived
43	3 c.c.	Lived	0.1 c.c.	x 3 days
44	3 c.c.	Lived	0.1 c.c.	x 3 days
59	3 c.c.	x 2 days	0.1 c.c.	x 3 days
61	3 c.c.	Lived	0.1 c.c.	x 3 days
63	3 c.c.	x 2 days	0.1 c.c.	x 4 days
66*	3 c.c.	0.1 c.c.	x 1 day
85	3 c.c.	x 2 days	0.1 c.c.	x 3 days
90*	3 c.c.	0.1 c.c.	x 3 days
94	3 c.c.	Lived	0.1 c.c.	x 3 days
95	3 c.c.	Lived	0.1 c.c.	x 2 days
96	3 c.c.	Lived	0.1 c.c.	x 3 days
97	3 c.c.	x 2 days	0.1 c.c.	x 3 days
98	3 c.c.	Lived	0.1 c.c.	x 2 days
99*	3 c.c.	0.1 c.c.	x 3 days

* No satisfactory toxin tests, owing to poor growth.

The 17 virulence tests with ascitic broth are given in detail contrasted with the toxin tests of the same strains in broth without ascitic fluid, as illustrative of the extreme sensitiveness of the diphtheria bacillus to slight changes in its nutrient medium. The overlooking of this fact is probably accountable for many of the conflicting statements in the reports of different investigators. Further work in this direction might have brought out finer comparative differences.

The result of this series of tests, carried on through so many transfers, and with so large a number of cultures, emphasizes strongly the unusual vigor and toxin production of the No. 8 laboratory strain, and the retention of these qualities for so long a time in an artificial medium is a striking illustration of the relative stability of the diphtheria organism.

NON-VARIABILITY OF DIPHTHERIA BACILLI.*

JANE L. BERRY AND EDWIN J. BANZHAF.

(From the Research Laboratory, Department of Health, New York City.)

In 1908 Goodman¹ reported a series of tests with diphtheria bacilli by which he claimed to have established the fact of a natural process of selection among diphtheria organisms, and stated that by taking advantage of this he had been able to obtain, from a strain showing medium acid production in glucose broth, a diverging series, leading to two opposite extremes with regard to acid production.

Starting with a single colony from a case of clinical diphtheria he inoculated a series of tubes of sugar-free infusion broth containing 1 per cent dextrose, incubated for three days at 37° C., and then titrated to determine the final acidity. From the cultures showing the highest and lowest acidity 15 tubes of glucose broth each were inoculated, incubated, and titrated as before. In this manner the two strains, called the High and the Low respectively, were now carried through 36 transfers, the culture showing the maximum acidity being the only one selected each time to carry on in the High Series, and the one with the minimum acidity in the Low Series, plating weekly to avoid contamination.

While at first some irregularity was noticed, Goodman states that he soon found a gradually increasing variation in fermenting power between his High and his Low strains, until at the end of his experiment he had as wide a divergence as between a diphtheria and a pseudo-diphtheria strain, leading on the one hand to a very high acid-producing organism, and on the other to an organism producing alkali in glucose broth.

On the basis of this work he suggests that the division into several distinct species is probably based on a misconception, and that all diphtheria-like organisms are probably only variants of the one species.

Winslow and Walker,² taking up the same problem, consider that an important factor in Goodman's work was probably the

* Received for publication April 8, 1912.

¹ *Jour. Infect. Dis.*, 1908, 5, p. 184.

² *Ibid.*, 1909, 6, p. 90.

progressive effect of environment, the organisms being exposed for transfer after transfer to high and low acidities respectively, and therefore likely to gain or lose tolerance to the acid reaction. They planned by excluding this factor to deal only with spontaneous variations. Selecting strains of the two types A and B of the paratyphoid bacillus of Schottmüller, they plated and fished 100 colonies from each into agar tubes.

As soon as these showed distinct growth each was transferred to a tube of 1 per cent glucose broth, grown 72 hours at 20° and titrated. Next, going back to the original agar slants, those fishings which had shown the highest and the lowest acidities in glucose broth were selected to carry on for the High and Low types of each organism, and from each of these, plated out, a series of glucose tubes were inoculated and carried on as before.

The conclusion of this experiment showed quite opposite results to those reported by Goodman with the diphtheria bacillus. With an occasional overlapping in acid production, each series varied around a distinct center, and in each there was a complete reversion to the original mean of the type, no apparent modification of character having been produced by the above process. The entire 538 cultures of the two paratyphoid types fall between the limits of 1.15 and 1.95 acid, yet in this narrow range are evident two distinct modes corresponding to the two apparently distinct paratyphoid types.

Buchanan and Truax,¹ following the method of Goodman, carried out a similar experiment with *Str. lacticus* but failed entirely to obtain fixed high and low acid races of this organism.

Glenn,² working with organisms of the proteous type, also found that selection of slight variation did not result in the production of any fixed change as to acid production.

Our work along these lines was carried out with two strains of diphtheria organisms selected from among the 100 diphtheria cultures which had been previously obtained from the throats of diphtheria patients for use in a different study, and which had all been cultivated for many transfers on artificial media. Each of

¹ *Jour. Infect. Dis.*, 1910, 5, p. 680.

² *Centralbl. f. Bakt.*, 1911, 6, p. 481.

these 100 strains was transferred to glucose broth, grown for three days in the incubator, and then titrated with phenolphthalein as an indicator, the results being tabulated according to acid production. A second test of the 100 strains was made in the same manner, and two strains were then selected for further experiment. One of these, known as No. 71, while always showing entirely typical organisms on Loeffler blood serum, grew with difficulty in broth when first isolated, producing no pellicle until after long cultivation, and showing dark, coarsely granular, atypical colonies on agar. This organism produced but little toxin in broth, requiring 0.1 c.c. to kill a small guinea-pig in four days, and the preliminary titrations showed but little acid production in glucose broth. The other strain, No. 51, was active in all respects, growing vigorously and typically from the beginning in all media, producing sufficient toxin in broth to kill guinea-pigs in two days with 0.01 c.c., and in six days with 0.005 c.c. and showing a high acid production in glucose broth. It was thought that by starting with these two strains, already at opposite poles of the scale of ordinary variation, there would be the most favorable opportunity offered for the development of Goodman's natural selection process.

The glucose broth used in the experiment was made in the chemical laboratory under Dr. Banzhaf's direct supervision, and the titrations were carried out by Miss Fisher and Miss Stryker under Dr. Banzhaf's direction. The broth was made as follows:

The clean muscle meat from young veal was ground up. Tap water was added (1 liter water to 1 pound of ground-up meat). An 18-hour colon culture in broth was added. This mixture was allowed to ferment about 20 hours at 22° C. It was then digested for two hours at about 55° C., boiled for 15 minutes, and the meat strained off. Two per cent peptone and 0.5 per cent of salt was added. The medium was then titrated using phenolphthalein as an indicator. Sufficient normal sodium hydroxide was added to adjust the acidity to 1.2 per cent normal acid. Boiled for 15 minutes and filtered clear.

The titrations were carried out in the original tubes of glucose broth culture. Each tube contained 10 c.c. of the medium. Phenolphthalein was used as the indicator. Decinormal sodium hydroxide was used to determine the acidity. The titration was carried on, against a white background, until a faint pink color appeared in the medium. With practice the faint pink color in the medium can easily be distinguished.

The two strains selected for the experiment were plated out on agar, and 100 colonies from each strain were fished on to the surface

No 77.

[illegible]

of agar slants over which 0.1 c.c. of ascitic fluid had previously been dropped from a pipette. The fishing needle was washed off in the water of condensation and ascitic fluid in the bottom of the tube, and this was then floated up over the surface of the agar. By this method a good general growth was produced over the surface of the slant, thus avoiding the usual irregular growth from fishings.

The agar cultures, after 24 hours in the incubator, were washed off with 1 c.c. of ascitic fluid into tubes of glucose broth which were grown in the incubator for three days and then titrated as above described.

The result of these titrations showed a different ratio of acid production to that given by the two strains in the preliminary tests, but as the two trials then made had agreed in showing high acidity in No. 51, and a low grade in No. 77, and because of their suitability in other respects, it was decided to go on with the experiment as planned, using these two strains.

Going back to the original agar tubes, which had been returned to the incubator, and again showed a good growth, the three fishings were selected which had shown the highest acid production, and also the three showing the lowest acid production in each strain. These were all plated out, and from 16 to 20 or more colonies of each kind fished into tubes of agar slants plus ascitic fluid, as before, thus averaging about 60 fishings each for the new High and new Low series in each strain. These were now transferred to glucose broth, grown and titrated as before, and again the three highest and three lowest selected for further use.

By proceeding in this way it was hoped to eliminate mere accidental variations, and to give the fullest opportunity for the influence of natural selection. No. 77 was thus carried through eight, and No. 51 through nine series of cultures and titrations, with occasional sets missing owing to breakage or contamination. The results are given in the accompanying table, where the figures show the number of fishings giving the same amount of acid in each set, and the connecting lines show the source of the successive groups.

It will be seen that throughout this experiment there is no evi-

dence whatever of the progressive development described by Goodman. Although there seemed at first some indication of a tendency toward division on the basis of acid production, this soon disappeared so completely that the most striking feature of the whole experiment, with both strains, was the tendency shown by the various fishings, not to diverge, but to approach each other in the degree of acid productions. The irregularity was marked, so much so that conditions were sometimes reversed between two series, the Low of one furnishing the High of the next succeeding series and vice versa, but at the end of the experiment all fishings were seen to be grouped within a very narrow range of acid production in each strain.

It may be said that results might have been different had we carried the experiment farther, but with the large number of substrains used at each transfer, together with the improved technic suggested by Winslow and Walker, it seemed to us that if a tendency to fixed variation exists in the diphtheria bacillus, there should have been at least some indication of it in our series, amounting to over 1,000 titrations for No. 77, and over 1,100 titrations for No. 51.

We cannot exclude the possibility that Dr. Goodman in his experiment may have worked with a mutating organism, but in the extensive work carried out for many years at the Research Laboratory no evidence has ever been found of the existence of such mutation among diphtheria bacilli. The result of the present experiment, moreover, seems to furnish another proof of the fixed characteristics and non-variable nature of pure cultures of the diphtheria organism.

Thanks are due to Dr. Park and Dr. Williams for advice and suggestions in this and the preceding study.

CONCENTRATION OF ANTISTREPTOCOCCIC AND ANTIGONOCOCCIC SERA.*

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Antistreptococcic sera and antigenococcic sera have been on the market for some time, but doubtful success, and in some instances, failure, have attended their use. The poor results may be due to several causes, among which low potency is possibly an important factor. We therefore concentrated these sera according to the methods used for the concentration of diphtheria antitoxin. It has been shown¹ that a serum for Rocky Mountain spotted fever can be concentrated by precipitating the pseudoglobulin fraction and that the antibodies are united chiefly to this fraction. The antistreptococcus sera for this purpose were obtained from horses immunized with 36 strains of streptococci under the direction of Dr. D. J. Davis and Professor H. G. Wells, and the antigenococcus serum was obtained through the courtesy of Parke, Davis & Co., in Detroit, Mich.

Diphtheria antitoxin is concentrated usually by the methods devised by Gibson and Banzhaf. The modification introduced by Banzhaf consists in heating the plasma, obtained by drawing blood into a solution of sodium citrate or potassium oxalate, for a period of about 15 hours at 56° C. The plasma is then diluted with about one-half its volume of water and enough saturated ammonium sulphate solution added to make a 30 per cent concentration. The precipitate is filtered off, redissolved in water, and the solution saturated with sodium chloride. This saturated solution is filtered and the globulins precipitated from the filtrate with 2.75 c.c. acetic acid per liter. The precipitate is then gathered on paper filters, pressed out to remove mechanically as much of the liquid as possible, and the precipitate, now forming a solid cake, dialyzed against running water. After the precipitate has gone into solution,

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¹ *Jour. Am. M. Ass.*, 1911, 57, p. 198.

enough sodium carbonate is added to make the reaction amphoteric. In about seven to nine days the ammonium sulphate and sodium chloride are dialyzed out and the globulin solution is filtered, first through pulp of filter paper and then through a Berkefeld filter for sterilization.

The filtrate remaining from the first precipitate is mixed with enough ammonium sulphate solution to make the concentration 55 per cent. The resulting precipitate is gathered on paper filters, pressed out, and dialyzed in the same manner as the first precipitate. In this case, however, it is not necessary to neutralize the globulin solution.

The loss of potency by heating antidiphtheritic serum previous to precipitation is slight. The advantages of heating are twofold. There seems to be a rearrangement of the relation of the constituents of the serum on heating so that a part of the superfluous protein is rendered insoluble and consequently a higher concentration of antibodies is obtained. Heat also destroys toxic substances which cause local reactions after injection. Serum rashes are less common if the serum has been heated previous to precipitation. We considered that it might be well, however, to try different methods of concentration, to be able to judge the effects by the results.

The tests for potency of antistreptococcic and antigenococcic sera were made by using the opsonic index, in default of more exact methods. The following routine was carried out in determining the potency of the original sera and the concentrated sera.

The leukocytes were obtained from blood drawn from the ear of the operator into a sterile centrifuge tube containing 2 per cent sodium citrate solution. The blood, mixed with the citrate solution, was centrifuged, and the citrate solution removed from the corpuscles by means of pipettes. The corpuscles were washed twice with physiologic salt solution and the clear fluid pipetted off. The leukocytes formed a thin white layer on the surface. The leukocytes were suspended in a small amount of physiologic salt solution. The bacterial suspension was prepared from 24-hour cultures on blood agar. A number of strains were tested and two selected which seemed to represent an average susceptibility to

phagocytosis. These two strains were used throughout the series of tests made. Clumps were removed from the suspensions by centrifugalization.

The opsonic index was determined by comparing the action of normal horse serum with serum from the horses immunized with streptococcus cultures. The normal serum was obtained from a horse treated with diphtheria toxin for the production of diphtheria antitoxin. The same horse was bled in all cases and at the same time, when the immune blood was drawn. The two sera were kept together under identical conditions. As the process of concentration takes about two weeks, the concentrated sera were compared with normal serum obtained two weeks previously and kept on ice in the meantime. The antigonococcic serum was compared with the same horse serum which was used in experiment 1. A laboratory strain of the gonococcus was used and the figure obtained (0.82) is of comparative value only, inasmuch as the strain used was different from the one used by the manufacturers for preparation of the serum. In determining the opsonic index of the concentrated sera it was found that the opsonic content was so high that a single cell would take up more organisms than could be counted with accuracy. For this reason the concentrated sera were diluted with salt solution. The opsonic preparation was made by using a Pasteur pipette. A mark was made on one of these pipettes and successive portions of serum, bacterial suspension, and leukocytic suspension drawn up to this mark, separating the various portions by air bubbles. The three portions were mixed, the tip sealed, and the preparation incubated for 20 minutes at 37° C. The contents were then spread evenly over a number of glass slides, dried in the air, fixed by gentle heating, and stained with methylene blue. In making a set of preparations the same bacterial suspension and the same leukocytic suspension were used for the whole series. The opsonic index was obtained by counting the bacteria found within 200 phagocytes. Four preparations were made of each serum, so that 800 phagocytes were counted for each test.

The first experiments with concentration were made with small amounts of antistreptococcic and antigonococcic serum. They

were not heated, and the first precipitate, which is relatively small, was discarded. The second experiment was made with anti-streptococcic serum only. This lot was not heated, but both precipitates were carried through. The third experiment was made with antistreptococcic serum and this was heated, and both precipitates carried through. The results appear in the following table.

No. of Experiment	Kind of Serum	Volume of Blood in Oxalate Solution	Volume of Plasma	No. of Concentrated Serum	Volume after Concentration	Opsonic Index before Concentration	Opsonic Index after Concentration	Concentration of Opsonins	Per Cent Yield of Opsonins Compared with Original Content	Loss in Filter
1...	Gonococcus		960 c.c.	61	150 c.c.	0.82	3.000	3.655		40 c.c.
	Streptococcus	8,000 c.c.	3,790 c.c.	1	500 c.c.	2.053	7.006	8.41	45	
2...	Streptococcus	10,000 c.c.	5,380 c.c.	2	850 c.c.	2.898	13.342	4.604		
				21	460 c.c.	2.898	5.963	2.058	67.5	275 c.c.
3	Streptococcus	16,000 c.c.	7,400 c.c.	31	670 c.c.	1.525	8.621	5.651	32	195 c.c.
				31	300 c.c.	1.525	5.353	3.509		

There is always some loss from manipulation in the process of concentration. This loss, however, can be reduced to a minimum by care in all details. The greatest loss occurs no doubt during filtration of the globulin solution. Considerable quantities are retained in the walls of the Berkefeld filter and a residue remains on the outside of the filter. Salt solution was passed through the Berkefeld filter after filtration of the sera. The filtered salt solution contained nearly all the serum left in the filter and this solution was incorporated with the next lot. In experiment 1 the amounts worked with were too small and we did not pass salt solution through the filter. In experiment 2 we found that the loss in the filter was 275 c.c. This amount was incorporated with the serum in experiment 3. The amount of loss was determined by deducting the filtrate from the amount obtained after dialysis. The incorporation of the remnant of experiment 2 with experiment 3 involves a slight inaccuracy in the opsonic index of serum S 3 and S 31. This inaccuracy, however, is negligible. The percentage of yield of opsonins is the amount calculated in relation to the opsonic content of the original serum. The relative loss of manipulation is larger if small amounts are worked with. In experiment 1 the

yield was 45 per cent. in experiment 2, 67.5 per cent. and in experiment 3, 52 per cent. The relatively low yield in experiment 3 is probably due to partial destruction of opsonins by heating at 56° for 15 hours.

The ratio of concentration is about the same as is obtained in concentrating diphtheria antitoxin. The antibodies in anti-streptococcic and antigonococcic sera seem to be distributed among the globulins in the same relation as in diphtheria antitoxin.

The results of this work show that antistreptococcic sera and antigonococcic sera can be concentrated by the same method as diphtheria antitoxin. The globulin solution may have a potency of 3 to 5.6 times the potency of the original serum, as measured by the opsonic index. We have also shown that in these sera the chief amount of antibody is united with the globulin fraction of horse blood, the same as in diphtheria and tetanus antitoxins, and with the antibodies to Rocky Mountain spotted fever.

The clinical efficiency of the concentrated sera is being tested and the results will be published later.

THE EFFECTS OF CHEMICALS ON THE DIVISION RATE OF CELLS WITH ESPECIAL REFERENCE TO POSSIBLE PRE-CANCEROUS CONDITIONS.*

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AND

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Three years ago one of us carried out some experiments on regeneration in single cells, using for the purpose the ciliated protozoon *Uronychia transfuga* St. It was found that the power of regeneration varies at different periods of cell life, being lowest immediately after division and greatest immediately prior to division. From these results the conclusion was drawn that the power of regeneration is bound up with the accumulation of some product of metabolism which reaches a condition analogous to saturation just before division and is exhausted by the process of regeneration involved in the reconstruction processes after division. This led to the further hypothesis that division itself might be bound up with the accumulation of some product of metabolism.

As the central problem of cancer research is connected with the exciting cause of cell division we decided to try out the hypothesis by some direct experiments. The obvious chemicals to employ in such a test are the products of nucleo-protein breakdown, including amino acids, nucleins, and their derivatives. In the present paper we wish to present some of the results obtained in these experiments which were conducted on single-celled organisms, and on the mature tissues of peritoneal organs of rats. For many of the pure chemicals used we are indebted to the courtesy of Dr. P. A. Levene of New York, and Dr. Walter Jones of Baltimore.

THE EFFECTS OF CHEMICALS ON THE DIVISION RATE OF ACTINOBOLUS.

One of the free living organisms used in the experiments is an extremely rare ciliated protozoon *Actinobolus radians* Stein. This form has an advantage over other types of protozoa for the present

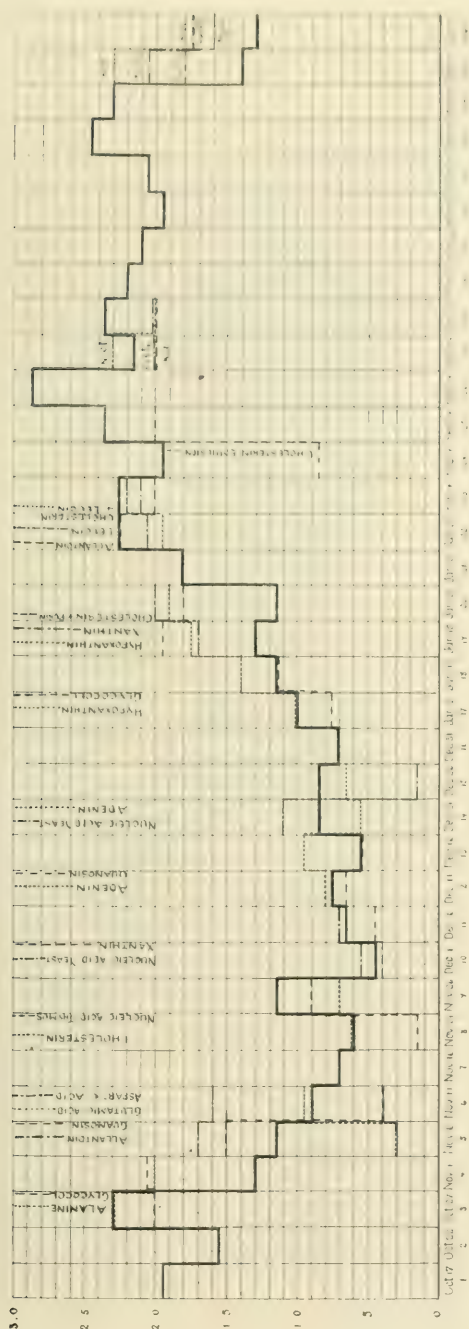
* Received for publication May 2, 1912.

work in that it is not a bacteria-eater but is restricted to animal food, ingesting no other kind of food than the protoplasm of *Halteria grandinella*, another ciliated protozoon.

One of these protozoa was captured and isolated in a few drops of filtered pond water on October 12, 1911. It was fed with *Halteria* and placed in a moist chamber. In a day or so it had divided several times and the daughter-cells were isolated in watch glasses, supplied with pond water and food as before. This procedure has been repeated daily since that time, the race now being in the 375th generation of cell divisions. Several pure lines were started, four of which have never been tampered with, and these have served as control lines for all of the experimental work. The history of these four lines, as shown by the average division rate, is shown in the accompanying diagram. In this the ordinates represent the average daily rate of division for each individual cell of the four lines; these in turn are averaged for periods of five consecutive days. The abscissae represent the successive five-day periods from October 17 until April 8.

The beginning of the curve on October 17 shows that during the first five-day period the individuals were dividing at the rate of 1.95 divisions per day. This rate is better expressed, for purposes of comparison, by the statement that the organisms were dividing at the rate of 19.5 divisions in 10 days, and this form will be followed in the sequel. In the second period the rate fell to 15.5, but rose again in the third period, when the first experiments were made, to a rate of 23 divisions in 10 days. From this high rate the rate of division declined in successive periods to the lowest rate reached during the course of the experiments, viz., 4.5 during the 10th period. From this low point the division rate slowly increased to a maximum of 28 divisions in the 26th period, in February, and this high rate was again succeeded by a gradually decreasing rate.

The control curve thus shows a characteristic rhythm of growth energy with one period of depression during November and December, a result which has been obtained with practically all similar cultures of protozoa, and a phenomenon which has been recognized in connection with various kinds of rapidly dividing cells, even in



cancer cells, according to the observations of Bashford, Hertwig and Poll, and others.

In the experiments, the individuals to be treated with chemicals were in every case daughter-cells of individuals of the control lines of the same date. The lethal dose of the chemical to be tested was first ascertained by experiment, and was then diluted to such an extent that neither *Actinobolus* nor its food *Halteria* was killed by 24 hours' exposure. The same quantity of pond water and food was used and every other condition was the same as with the control lines, the only difference being the addition to the medium of the chemical experimented with. Four lines of cells were used in every test and four different strengths of the chemical. The tests extended in every case over a period of 10 days, thus giving two five-day periods for comparison with the control. For this comparison we have used only the average of the four experimental lines; in many instances more marked differences between the experimental and the control series would have been obtained had we used only that strength of chemical which gave the most pronounced reaction.

For the best analysis of the results we will compare the reactions of the chemicals at different periods of vitality of the protoplasm in the control lines. Four such periods are evident from the curve: first, a period of decreasing vitality as indicated by a rapidly descending division rate from October until the end of November (periods 1-9 inclusive); second, a period of low vitality during the month of December (periods 10-16 inclusive); third, a period of increasing vitality from the first of January to the end of February (periods 17-26 inclusive); and fourth, a period of decreasing vitality from the end of February to the middle of April (periods 27-36 inclusive).

Effects of chemicals during the first period of decreasing vitality.—During this period eight chemicals were tested: alanin, glycocoll, allantoin, guanosin, glutamic acid, aspartic acid, cholesterin, and nucleic acid from the thymus. Alanin and glycocoll were used with a maximum strength of $\frac{N}{10}$, one drop to five drops of food medium. During the two successive five-day periods under treatment the

control lines dropped from a rate of 23 divisions in 10 days to a rate of 13 divisions. The sister cells treated with alanin divided the same number of times as the control during the first period and the rate then fell to 20 divisions, or seven better than the control. Those treated with glycocoll maintained a steady rate of about 20 divisions in 10 days, throughout the experiment. These mono-basic amino acids, therefore, appear to have had the power of sustaining vitality when the division rate of the unstimulated protoplasm was rapidly falling. That the action was not due to these complex substances acting as food was proved by the simple experiment of omitting the Halteria from the food medium and using only pond water with the chemical. In the specimens thus treated the division rate was the same as that of the control for the first 24 hours but after this not one of the individuals divided again and all died from starvation within 72 hours.

The purins guanosin and allantoin were used in the form of saturated solutions. This, for allantoin, was diluted 1:10 and used in the maximum strength of one drop to five. Guanosin, diluted 1:5, was used in the maximum strength of one to four. Specimens treated with allantoin divided at the rate of 17 and 16 divisions in 10 days, while those treated with guanosin divided at the rates of 15 and 4 for the two periods of five days each. The control lines divided at the rates of 11.5 and 9. It is significant that the greater effect of stimulus is shown by allantoin, a later product of nucleo-protein breakdown.

A well marked depressing effect was produced by the dibasic monamino acids, glutamic, and aspartic, the rates for which were 3 and 9 for glutamic, and 3 and 4 for aspartic, as against 11 and 9 for the control. These acids in saturated solutions were diluted 1:20, 1:40, 1:80, and 1:160 and used one drop to five.

Cholesterin and nucleic acid from the thymus, used in saturated solutions diluted 1:30, one drop to five, had but a slight effect on the division rate at this time.

Effects of chemicals during the period of low vitality.—During the periods from 10 to 16, the division rate of the control averaged a little less than 7 divisions in 10 days, the lowest rate for a five-day period being 4.5, the highest 8.5 divisions in 10 days. The

chemicals used were nucleic acid from yeast (periods 10 and 11), xanthin (periods 10 and 11), adenin (periods 12 and 13), and guanosin (periods 12 and 13). Saturated solutions were used in all cases. During these periods the nucleic acid and xanthin had little appreciable effect. Adenin had a curious effect, giving a slight stimulus in periods 12 and 13, and a depression in periods 14 and 15. Guanosin, also, had but slight effect, if any, at this period. With these chemicals at this time, therefore, the variations from the control are too minute and too indefinite to warrant conclusions.

Effects of chemicals during a period of increasing vitality.—During this third period of the history of *Actinobolus* the division rate rose from a low rate of 7 divisions in 10 days in the first period in January (period 16) to a maximum rate of 28 divisions in the fourth period in February. The increase was steady during this time and has as steadily declined since then.

The most interesting results at this time were obtained with the chemicals hypoxanthin and xanthin, both in saturated solutions diluted 1:100 and 1:80 respectively. Examining the action of hypoxanthin first the control curve shows that both control and chemically treated individuals were dividing at the rate of 10 times in 10 days. In the second period of treatment (period 18) the relation was 11.5 control and 14 hypoxanthin. In the third period (19) the relation changed to 13 and 17.5 respectively, while in the fourth period the control fell to 11.5 and the hypoxanthin-treated cells rose to 19.

The individuals treated with xanthin showed a similar stimulation, the control dividing at the rate of 13 divisions in 10 days (period 19) during the first period while the treated cells rose to 17. The difference was still more manifest in the second five-day period (20) when the rate for the control fell to 11.5 and that of the xanthin-treated individuals rose to 20. When this result is contrasted with that obtained with xanthin during the period of depression in December (periods 10 and 11) when a still greater depressing effect was produced by the chemical, the comparison is interesting and significant and indicates that the factor of vitality must be taken into consideration in all experiments on the effects of chemicals.

A similar high division rate was obtained with cholesterol mixed with xanthin and hypoxanthin. The potency of the mixture being due, presumably, to the action of the purins, for cholesterol when used alone had a depressing effect on the division rate (see periods 8, 9, and 24, 25).

Allantoin, which had been found to have a stimulating effect when the protoplasmic vigor was declining, was again tried when the rate was high (22.5 divisions). There was no effect during the first five days, the division rate remaining the same as the control, but in the second five-day period the individuals treated with allantoin fell to a rate of 20 while the control remained the same (periods 22 and 23). Leucin, tested at the same time, brought the rate down to about 20 divisions for the 10 days.

Summarizing in a word the results on free living cells, there seems to be little doubt that, under certain conditions of protoplasmic activity, some chemical products obtained by the hydrolysis of nucleo-proteins have a definite stimulating effect upon the division rate of free living cells. At the present time we have no hypothesis to offer as to the interpretation of the chemical reactions involved nor do we wish to make any attempt to harmonize our initial working hypothesis with the results obtained. It is certainly significant, however, that the later products of the hydrolysis of nucleins have the greatest stimulating effect upon the division rate.

THE EFFECTS OF CHEMICALS ON TISSUE CELLS.

There are many difficulties to overcome in getting a successful local application of chemicals on tissues in the living body. The agar-agar method, which we finally adopted, is an adaptation of one described by Leo Loeb in 1905. This method has the advantages of permitting a uniform mixture of the chemical; of allowing the permeation by new cells in their growth, thus enabling them to come in direct contact with the chemical; and of firmly holding insoluble or slightly soluble chemicals, thus preventing or retarding their removal by phagocytes and prolonging their chemical action.

The chemical or chemicals to be tested were thoroughly mixed in a 2 per cent agar jelly. Of this mixture 2 c.c. were injected

while the agar was still liquid (usually at a temperature of about 44° C.) in the subcutaneous regions of the breast or in the peritoneal cavity of normal rats. In all experiments, except when otherwise specified, 0.5 gm. of the chemical was mixed in 15 c.c. of agar.

For each experiment sometimes four, sometimes five adult animals were used and killed at different intervals; the injections were invariably made under ether anaesthesia and the animals were always killed while under ether. Complete autopsies were made in every instance and all tissues showing changes in the gross were carefully fixed in sublimate acetic or some other equally good cytological fixing fluid.

The chemicals were used either in combination with cholesterol or alone. Those used with cholesterol are listed on p. 430; those used alone included the following: cholesterol, glycerin, skatol, methylglycin, tyrosin, amido-acetic acid, creatin, cystin, leucin, sodium amido formate, asparagin, aspartic acid, ammonium carbamate, alloxanthin, hippuric acid, lecithin, guanin, guanin hydrochloride, xanthin, uric acid, and urea.

As the results after subcutaneous and intraperitoneal injection involve somewhat different reactions they may be more conveniently described under the headings "Subcutaneous Reactions," and "Intraperitoneal Reactions."

Subcutaneous reactions.—In this series of experiments all of the single chemicals enumerated above were used with agar. The dosage was the same in the case of all chemicals except guanin hydrochloride in which it was reduced to 0.25 gm. in 15 c.c. of agar.

In all cases the gross pathological changes are the same. After 10 days' exposure the injected material is surrounded by a firm, dense capsule varying in thickness from 0.5 to 5.0 mm. Ingrowing strands of connective tissue cells invade the agar in all directions. With longer exposure these strands become more dense and more firmly knit until by the 30th day the mass of agar in some cases is quite replaced by newly formed tissue.

A typical illustration of the microscopic picture is given by the reaction following injection of agar asparagin. At the end of 10 days the agar is largely replaced by good sized cells of rounded or

polyhedral form and with indefinite margins. These may contain one rounded or ovoid nucleus or there may be many such arranged about the periphery. The cytoplasm takes a deep acid stain with eosin and may inclose fragments of agar. Mitoses are frequently seen. These giant cells, possibly derived from polyblasts, are numerous and closely packed together in some areas, being separated only by a thin stroma of connective tissue cells. In some areas, particularly at the periphery of the agar, such giant cells may fuse, forming syncytia of various sizes and of fantastic shapes, and all with indefinite cell walls. The relatively few nuclei of such masses are arranged about the periphery. The development of these syncytia has been completely worked out by Mallory, Wolbach, and others, and need not be considered further.

The deeper portions of the agar are invaded by leukocytes, polyblasts, and other wandering cells, which become reduced in number, however, with longer exposure. In short, the result may be regarded as a granulation tissue reaction with capillary, fibroblast, and giant cell formation. The connective tissue is finally condensed into a firm capsule while the agar is more and more organized by the ingrowth of cells, with syncytia and giant cells (which sometimes show hyalin and fatty degeneration) distributed here and there.

Similar pictures are produced by guanin, guanin hydrochloride, alloxanthin, tyrosin, creatin, sodium amido formate, leucin, amidoacetic acid, aspartic acid, methylglycin, uric acid, hippuric acid, and urea. Ammonium carbamate causes necrosis of tissue as a result of which the early changes were not observed. With skatol and cystin the early reaction persists throughout. Lecithin produces a milder reaction, the late stages described above not appearing. Cholesterin causes considerable necrosis and degeneration of the tissues, the early changes resembling those described, but scattered through the newly formed tissue are cholesterin crystal spaces around which cholesterin crystal giant cells have formed.

Intraperitoneal reactions.—In these experiments the following chemicals were used singly and in the same strength as with the subcutaneous injections: ammonium carbamate, asparagin, cystin, hippuric acid, uric acid, urea, alloxanthin, and xanthin. Com-

binations of single chemicals with cholesterin were also used in the proportion of 0.25 gm. chemical, 0.25 gm. cholesterin thoroughly mixed in 15 c.c. of agar. The chemicals thus used were: nucleic acid, nuclein, alloxanthin, xanthin, uric acid, urea, amido-acetic acid, ammonium carbamate, aspartic acid, cystin, creatin, asparagin, tyrosin, leucin, and hippuric acid.

Again the gross pathological changes are practically the same in all cases. The injected material forms masses of different sizes and shapes scattered throughout the peritoneal cavity. These are abundant on the upper surface and between the lobes of the liver; on the parietal surface of the spleen; around and between the irregularities of the pancreas, and on different portions of the stomach, intestines, and omentum. The deposits never extend beyond the peritoneal cavity. In animals killed shortly after injection, the agar is loosely attached to the various organs like a mantle; later it becomes firmly attached to the organs and is invaded and enmeshed by newly formed tissues.

The microscopical pictures resulting from the reactions differ according to the tissues involved. For purely descriptive purposes we will describe them under the headings "Peritoneal Reaction," and "Epithelial Reaction."

a) *The peritoneal reaction.*—The reactions produced by different chemicals differ in degree rather than in kind. In some cases giant cell and connective tissue formations are well marked; in other cases this particular reaction is slight (Pl. 2, Fig. 1). In general it may be stated that the peritoneal response is similar to that of the subcutaneous tissues, the essential difference being the unusual proliferation of mesothelial cells in the former, of connective tissue cells in the latter (Pl. 2, Fig. 2).

The mesothelial cells are arranged in layers around the fragments of agar, or invade the agar mass, forming a mesothelial network (Pl. 2, Fig. 3). These cells usually have an indefinite outline and are spherical, discoid, or columnar in form. Large and small syncytia, often of fantastic shape, are likewise formed within the agar mass. These also have an indefinite outline; their protoplasm stains deeply with eosin, and contains relatively few nuclei. Usually such giant cells inclose a particle of agar (cf. Pl. 2, Figs. 1

and 2). Sometimes there is a connective tissue capsule about several foci of reaction thus forming a tubercle-like mass.

In animals killed after 30 days there remains a more or less dense and fibrous structure containing various cellular elements and fragments of agar surrounded by giant cells, many of which are undergoing fatty and hyalin degeneration.

b) The epithelial reaction.—The epithelial reaction has to do with the response of connective tissue and functional cells of the peritoneal organs. Partly through the ingrowth of peritoneal connective tissue and partly by proliferation of the stroma of the glands, specialized secreting cells are cut off as islands or long strands from the bulk of the gland. Such isolated cells are usually normal but they may appear in various stages of degeneration. This reaction is quite characteristic of all of the intraperitoneal work, occurring not only when chemicals are present, but also with plain agar controls.

With certain chemicals, used in combination with cholesterin, notably with leucin, ammonium carbamate, uric acid, and urea, the stimulus to cell division is carried not only to the connective tissue elements of the organ but to the secreting cells themselves, thus indicating a far-reaching influence on cellular metabolism (Pl. 2, Fig. 4). This remarkable effect is most striking in the case of the supposedly fixed cells of the liver and pancreas. Innumerable mitoses of the epithelial cells are found in and around areas of granular degeneration involved in the connective tissue reaction. The majority of the mitotic figures are perfectly normal, although here and there degenerating forms may be found. This proliferation probably indicates an attempt on the part of the organ to regenerate. In the liver new bile ducts are formed, from the cells of which or from old gland cells, new liver cells are derived. Such newly formed cells may be found, surrounded by connective tissue, at a considerable distance from the bulk of the organ. In the pancreas marked changes in the histological picture result (Pl. 3, Figs. 5 and 6). In many cases the gland cells, cut off by the invading connective tissue, undergo what may be termed degenerative metaplasia, and become smaller, and flattened or cuboidal, completely losing their characteristic appearance (Pl. 4, Figs.

7 and 8). In other cases the individual cells are less affected, but the acini are irregular in shape and size (Pl. 4, Fig. 8). In such cells mitotic figures are abundant, two or even three in one field of the immersion lens being not uncommon (Pl. 4, Fig. 9). In both liver and pancreas these specific reactions occur mainly in the region of the agar deposit and only rarely in distant parts of the organ.

In addition to the general reaction described above, some of the chemicals produce changes different from others or more marked in extent. With urea there is a clearly defined local degeneration of liver cells together with numerous mitoses, chiefly in the vicinity of the injected mass but evident also in distant parts of the organ, Pancreas cells on the other hand are not thus affected. With leucin, in all of the organs examined, there are in some cases peculiar and highly characteristic reactions analogous to the changes during acute yellow atrophy in the human. There is a widespread degeneration of all the visceral organs, the parathyroids alone excepted; the liver cells are undergoing more or less fatty degeneration, and in the organ as a whole there are innumerable areas of focal necrosis, surrounded by actively proliferating liver cells and newly forming bile ducts. With ammonium carbamate and uric acid the changes are sufficiently described in the general account given in the preceding paragraph.

ANALYSIS OF THE TISSUE EXPERIMENTS.

It is a matter of common belief that mitotic figures in glands like the pancreas and liver are extremely rare. Some authorities, indeed, question the occurrence of mitosis in fully developed normal cells of either organ. A number of questions now arise which demand consideration.

Are there analogous reactions in normal rats?

After finding so many mitotic figures in the experimental animals we began to doubt the traditional belief as to their scarcity in liver and pancreas. To convince ourselves, 10 supposedly normal rats were killed and sections from different regions of the pancreas and liver were made and carefully studied. Of these 10 rats six had parasitic cysts (cestode) of the liver which in every

case showed some degree of degeneration; and four showed a parasitic infection of the pancreas, the exact parasite not being identified. Mitotic figures in the pancreas cells were not found in animals free from parasites, but were occasionally found in the four infected animals, a search of from 15 to 20 minutes being necessary to find one. Mitotic figures in liver cells were somewhat more common, being occasionally present in all of the animals but more numerous in the infected ones.

The liver reaction to parasites deserves a little further consideration. Among the hundreds of rats autopsied 30 per cent were found to be infected, as shown by the presence of liver cysts. The parasite lies in a mass of viscous mucous-like fluid inclosed by a relatively thick connective tissue capsule containing few blood vessels and many plasma cells, some round cells, and leukocytes (Pl. 5, Fig. 10). In the walls of the capsule there are numerous newly formed bile ducts and strands of new liver cells from one to several layers thick. Some of these liver cells are in active mitosis.

From this examination of supposedly normal rats we may conclude, therefore, that mitosis in the pancreas occasionally occurs as a mild regenerative response to injury from parasites. Mitosis is more common in liver cells, presumably as a result of injury by parasites. In general, however, the number of mitotic figures does not compare with that found in these organs in the experimental animals.

Does agar without chemicals induce the same changes?

To determine whether the well marked responses are to be explained as exaggerated foreign body reactions, seven normal rats were given intraperitoneal injections of 2 c.c. of 2 per cent agar, the animals being killed at periods corresponding to those of the chemical series. Parasitic cysts in the liver were found in three of the animals. The gross changes were found to be the same as those already described. The peritoneal reaction also was similar, but the number of syncytial cells was much smaller (Pl. 5, Fig. 11). The characteristic delamination of liver cells was present in several instances but was not found with pancreas cells. Mitotic figures were comparatively rare, none being found in two livers and one pancreas, and only occasionally in the others. In no case

was there evidence of the so-called regenerative process found in the chemically treated animals. The picture, in short, shows no changes apart from the peritoneal reaction, different from our supposedly normal non-injected animals.

Do foreign bodies in the agar act as mechanical stimuli?

If the reactions set up are due to mechanical stimulation, any foreign body with the same physical characters as the chemicals employed should produce a similar result. To test this possibility, pure carbon and powdered silicon were each injected into normal rats, four animals receiving 2 c.c. each of agar carbon in the proportion of 0.25 gm. carbon to 15 c.c. agar, and four receiving an equal dose of powdered silicon. The gross changes were the same as before and the histological picture was practically the same as that produced by plain agar, giant cells, however, being more abundant. The results of the experiment gave no evidence to indicate that a purely mechanical stimulus is responsible for the deeper reactions in the chemically treated animals.

Do artificial products of nucleo-proteins act as stimuli?

It is a significant fact that the most effective chemicals employed to stimulate cell division, both in free living *Actinobolus* cells and in tissue cells are the later products of nucleo-protein hydrolysis, many of these being purin derivatives (xanthin, hypoxanthin, allantoin, uric acid, ammonium carbamate, and urea). Some of these are readily soluble and it is a question how long they actually act on the tissues before dissolving out of the agar.[†] They all produce cell degeneration and cell death in varying degrees.

The question now arises, *Do the products of cell autolysis give similar results?*

Another series of experiments was carried out in the hope of getting some answer to this question. In three normal animals the splenic end of the pancreas was tightly ligatured and left *in situ*. These we will designate Group A. In three other animals, forming Group B, the end of the pancreas was similarly tied and cut off distally to the ligature. In three other animals, comprising Group C, burnt pancreas tissue was mixed with agar and injected into the

[†] Control experiments with highly soluble dahlia were carried out to determine how long the agar might hold the chemical. Agar deeply colored with dahlia was injected into rats. One died at the end of the fourth day. The agar removed from the peritoneal cavity had lost considerable color, especially around the margins, but the color was by no means all gone, showing that some of the dahlia remained.

peritoneum. One animal of each group was killed on the third day; another was killed (or had died) on the sixth or seventh day, and the third was killed on the 10th day. The gross changes at autopsy do not concern us here. The reaction in rats injected with burnt pancreas and agar (Group C) was in all respects similar to that produced by plain agar alone. In Group B, with pancreas tied off and excised, there was a narrow zone of complete necrosis distal to the ligature and a zone of pancreas cells of about equal width in partial necrosis proximal to the ligature (Pl. 6, Fig. 12). In the zone of pancreas cells adjacent to the latter the following changes were observed: (1) a marked proliferation of the mesothelial elements of the pancreas and of the interlobular and interacinus connective tissue cells; (2) an immigration of leukocytes, plasma cells, and a few mast cells; (3) a shrinkage of the acini and partial degeneration of their cells; (4) loss of the compact organ structure with distinctly separated, irregularly shaped, or distorted acini lying in a reticulated tissue; (5) the presence of double nucleated cells, and cell inclusions bearing a striking resemblance to the cell inclusions found in human carcinoma (Pl. 6, Fig. 14). These cell inclusions were very abundant, as many as six being seen in one section of an acinus. Mitosis in this area occurs, but was not frequently found (Pl. 6, Fig. 13).

In the area adjacent to the last, minor evidences of degeneration were found, especially in connection with the cell membrane, zymogen granules, and chromatin, some of the nuclei, even, taking an acid stain. Here also, there was distortion of the acini, but there was an abundance of mitotic figures.

In Group A, the reaction of the pancreas cells was identical in nature with that of Group B, but the changes were much more extensive and more decisive (Pl. 6, Fig. 15, and Pl. 7, Figs. 16 and 17).

In these experiments, therefore, we have evidence that the presence of cells undergoing autolysis produces the same type of epithelial reaction as that produced by pure chemical products of nucleo-protein hydrolysis. The failure to get the same reaction with burnt pancreas indicates that it is induced by active chemical substances from degenerating protoplasm and not by products of similar protoplasm killed by heat.

GENERAL DISCUSSION.

There has always been a tendency to associate cancer with the biological phenomena of regeneration. This is particularly well brought out in the *Fourth Scientific Report* of the Imperial Cancer Research Fund, in which it is argued that reparative processes following injury and under conditions of chronic irritation may become habitual, resulting in tumor growth. The contributors to this report emphasize the difference between the genesis of cancer and its continued growth. It is not improbable, however, that the factors at bottom are the same.

Our work on tissues has been done with the idea of getting light on the factors having to do with the genesis of cancer. The fundamental cancer problem, like that of regeneration, is expressed by the question: What induces the unusual division of apparently normal cells? The results obtained thus far demonstrate that we can induce mitosis by artificial means in tissues as stable as pancreas and liver, while a remarkable responsive development of mesothelium and connective tissue shows that the reactions involved are not limited to one type of cells, and that they follow the lines of normal regenerative processes.

We are accustomed to look upon unusual mitoses or regenerative processes as responses to stimuli. We know cases where metabolic products of parasites serve as stimuli to such development; for example, crown galls and vegetable galls in general. Such products are toxins belonging probably to the group of nucleo-proteins.

By using products of similar nature in the pure state and free from bacterial contamination, we have produced innumerable mitoses, and other marked changes in the histological picture of tissues ordinarily regarded as fixed, viz., pancreas and liver. We have done in the body what many investigators are successfully doing with epithelial and connective tissue cells on artificial media outside of the body.

We have not produced a tumor—we should like to say that we have not yet produced a tumor—but we have produced, by known means, characteristic changes in normal tissues which might well

represent, and which may yet turn out to be, precancerous conditions.

Every student of cancer has his working hypothesis and there are many theories under which cancer work is progressing throughout the world. They all fail, however, to account for the essential factors in the problem, viz., the origin of the stimulus to division of the latent cell, and the sources of stimuli to the continued proliferation of cells during continued growth, metastasis formation, and transplantation. The advocates of the parasite hypothesis exhaust their energies in a vain search for the parasite. None has been found, while, on the other hand, many intracellular parasites are known, especially the gregarines and coccidia, which have no stimulating effect on the host cells. Advocates of the Cohnheim theory must still explain the sudden stimulus to division of cells of the questionable "embryonic rests," and their explanation would then apply with equal force to the latent normal tissue cells; the same is true of Ribbert's theory. Those who maintain that cancer is due to a change in the biological properties of cells are undoubtedly correct, but here, again, is the same unanswered problem: What induces the change? Theories of fertilization or of rejuvenation of epithelial cells have come and gone, unsupported by fact, and with far-fetched maturation analogies. Those who hold that cancer is due to changed mass relations of nucleus and cytoplasm are working in biological darkness and are unable to see either beginning or end of the cancer problem. Those who gravely state that cancer is due to cells which have lost the habit of function and acquired the habit of growth, although biologically justified, are hopelessly off the firing line, and come dangerously near those pessimists who insist that the cancer problem will be cleared only when the problem of life is solved.

Our experiments on protozoa and on mammalian tissues have been carried out under the stimulus of a more general, more neglected, and, as we believe, a more probable hypothesis than any of the above; this may be outlined somewhat as follows: as a result of abnormal, local, metabolic conditions, brought about by injury, by chronic irritation, by parasites, or by other causes, products of

autolysis are formed which stimulate the division energy of latent cells. This is ordinarily held in check by the regulatory processes of the organism, but with continued irritation the division energy outruns the regulation of the organism and a tumor results. The development of cancer, with the inevitable train of degenerating products, overrunning the regulatory control of the organism, is continued through the activity of the cumulative products of cell autolysis, the degeneration of cancer cells thus producing the stimulating agents for further and more widespread development.

On this theory we find it possible to interpret all kinds of tumors. The obvious weak point is the transition from a reparative process subject to the control of the organism, to a malignant process uncontrolled and unregulated.

EXPLANATION OF PLATES.

PLATE 2.

FIG. 1.—General peritoneal reaction following the injection of agar cholesterin urea. Various stages in the formation of syncytial cells (2).

FIG. 2.—General peritoneal reaction following injection of agar leucin (15 days). *O*, capsule of connective tissue around unorganized agar mass (*P*), *Q*, syncytial cells.

FIG. 3.—Peritoneal reaction following injection of agar cholesterin leucin (15 days). *E*, degenerating liver cells; *D*, proliferating liver cells in suspensory ligament.

FIG. 4.—Epithelial reaction following injection of agar cholesterin leucin (10 days). *A*, liver cell in mitosis.

PLATE 3.

FIG. 5.—Epithelial reaction of pancreas following agar cholesterin urea (15 days). *U*, normal pancreas acini; *V*, isolated acini undergoing degenerative metaplasia; *W*, remains of agar.

FIG. 6.—Peritoneal reaction involving displaced pancreas tissue following injection of agar cholesterin urea (20 days). *A*, island of pancreas tissue showing invasion of connective tissue (*B*); *C*, isolated acini.

PLATE 4.

FIG. 7.—Epithelial reaction following injection of agar cholesterin urea (15 days). *R*, area of degenerative metaplasia; *S*, agar masses; *T*, area of normal pancreas.

FIG. 8.—Epithelial reaction following agar cholesterin urea (10 days). Portion of pancreas showing change from acinous to duct type, with three mitotic figures (2).

FIG. 9.—Epithelial reaction following injection with agar cholesterin uric acid (15 days). Pancreas acinus with three mitotic figures (1).

PLATE 2

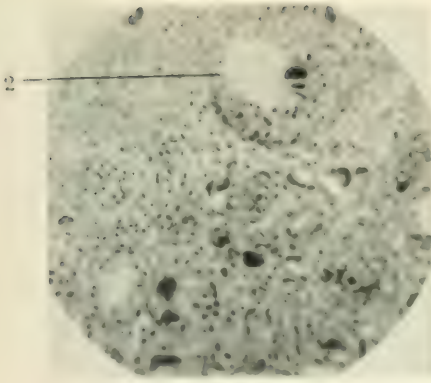


FIG. 1.

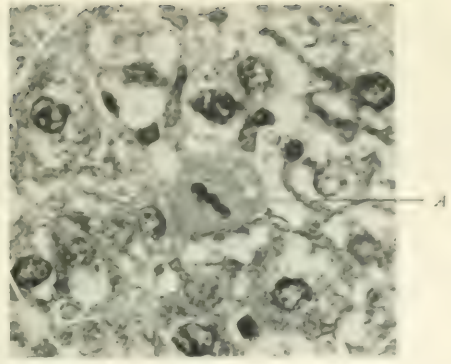


FIG. 4.

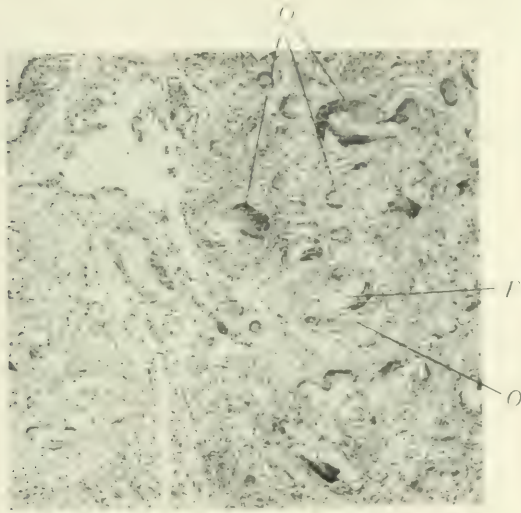


FIG. 2.

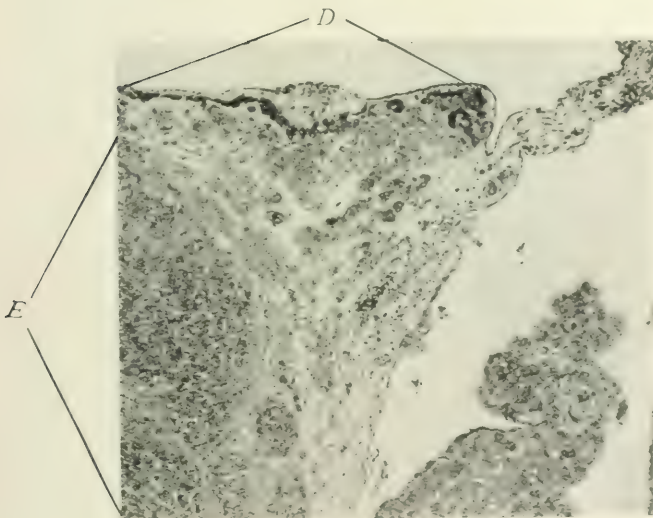


FIG. 3.

PLATE 4.

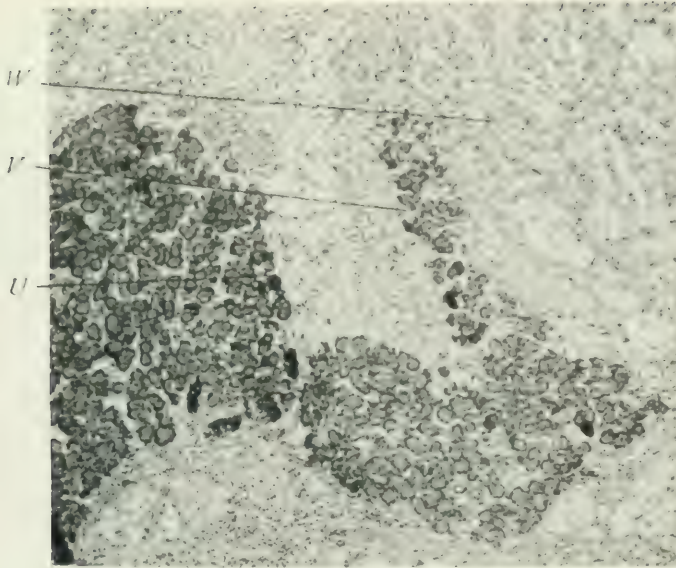


FIG. 5.

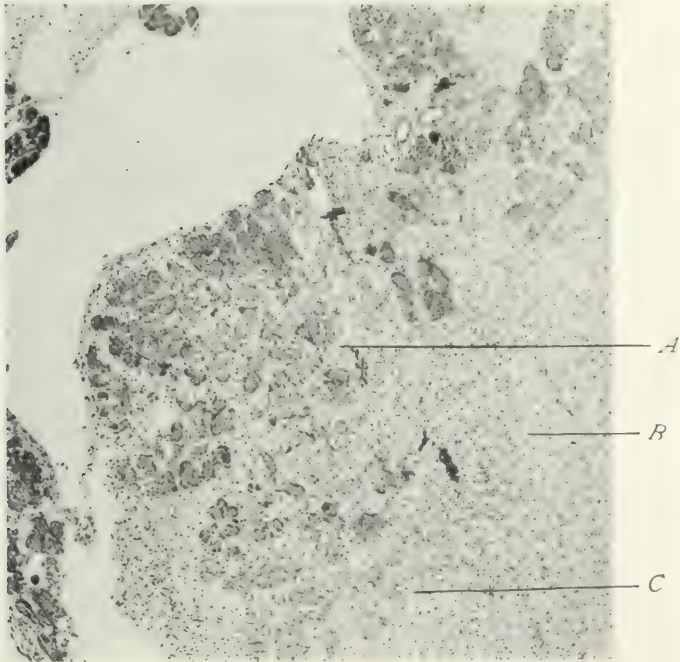


FIG. 6.

PLATE 4.

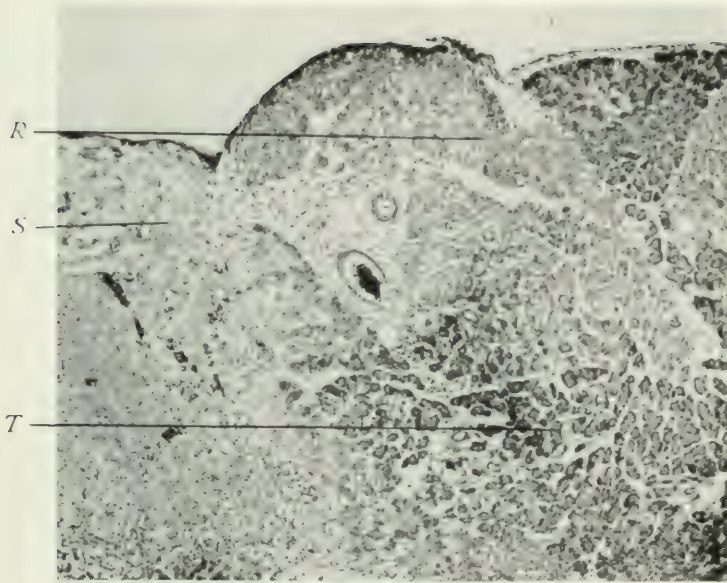


FIG. 7.

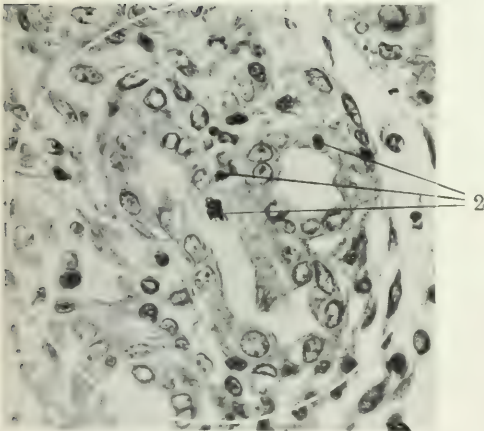


FIG. 8.

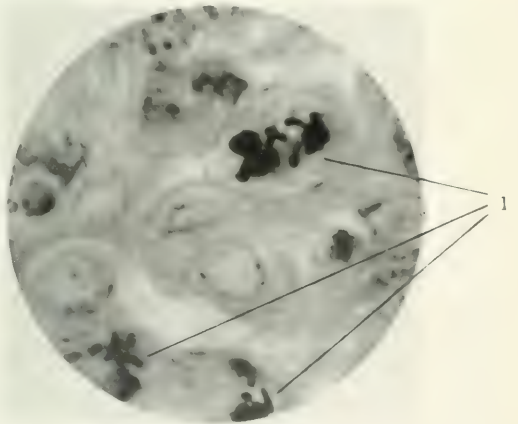


FIG. 9.

PLATE 3.

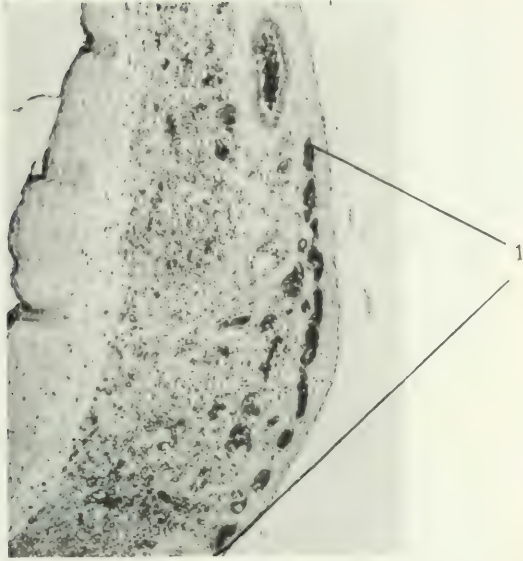


FIG. 10.

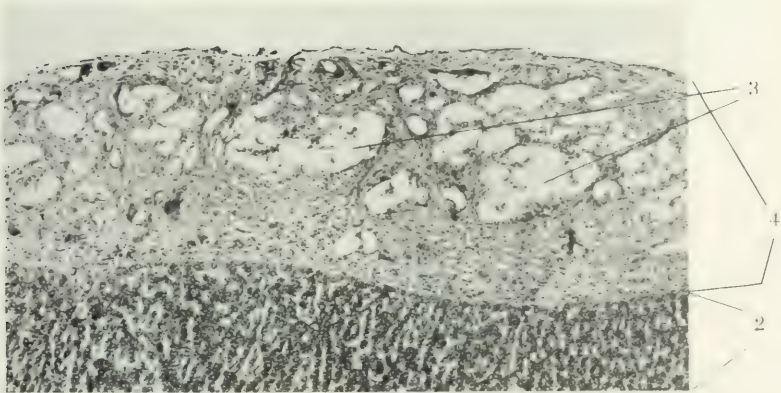


FIG. 11.

PLATE 6.

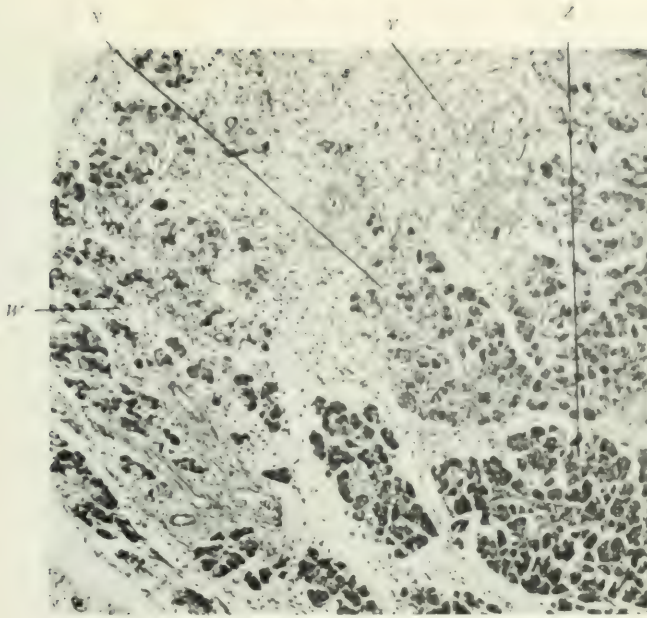


FIG. 12.

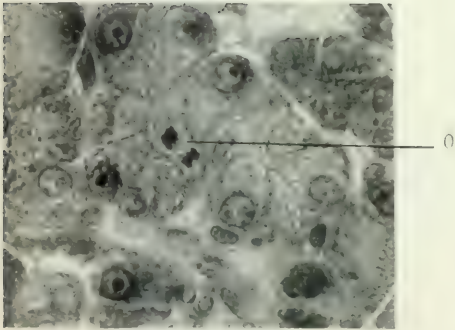


FIG. 13.

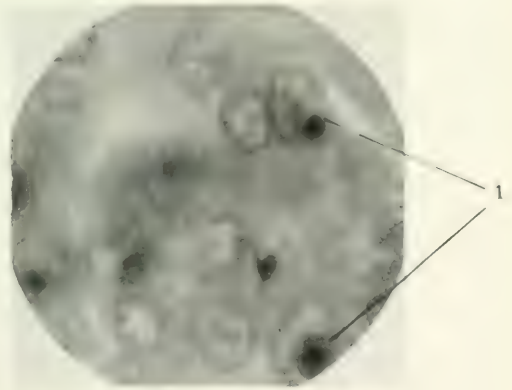


FIG. 14.

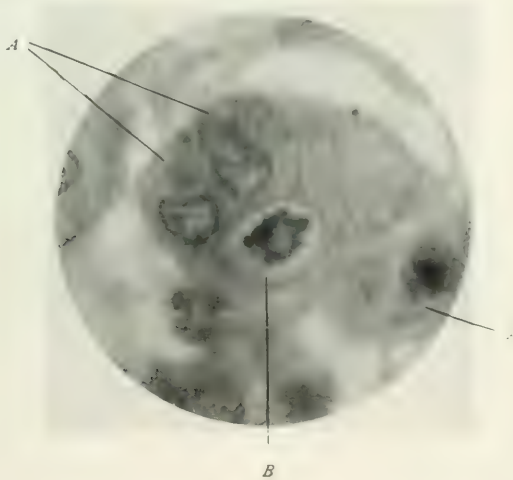


FIG. 15.

PLATE 7

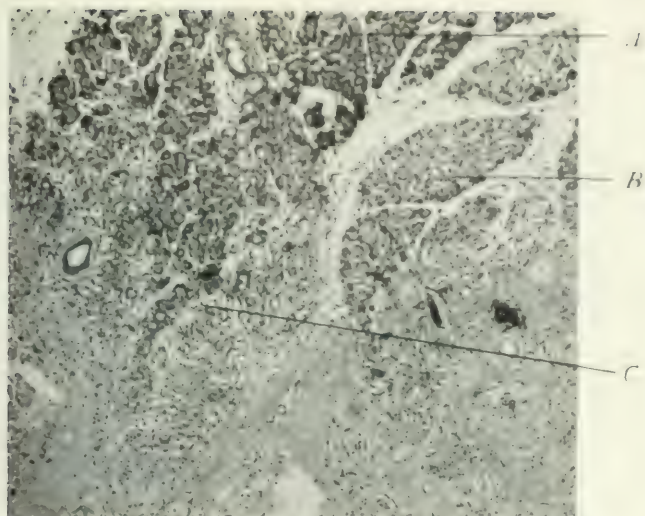


FIG. 16.

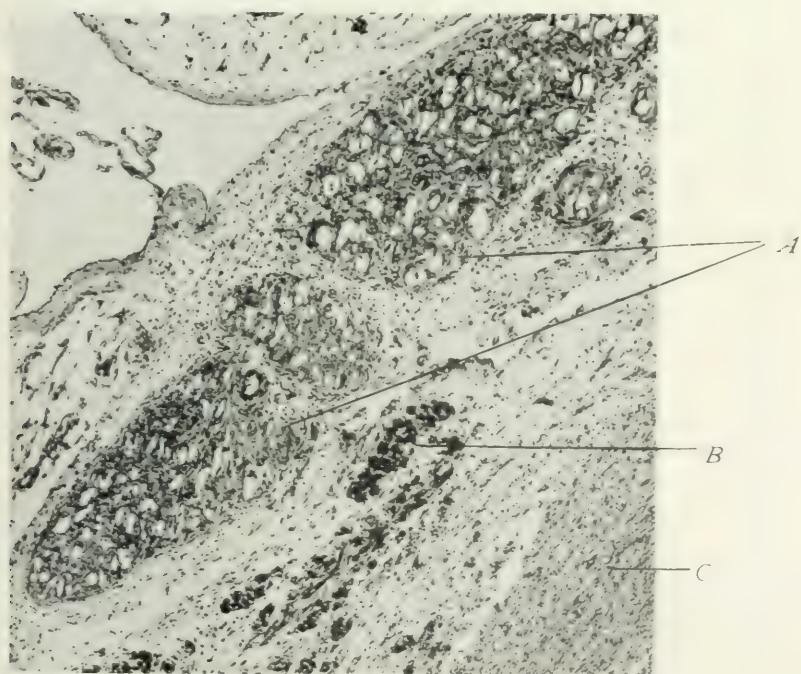


FIG. 17.

PLATE 5.

FIG. 10.—Wall of tissue of capsule surrounding potentio liver cyst. *a*, stratum of proliferating liver cells.

FIG. 11.—Peritoneal reaction following injection of plain agar. *a*, liver tissue; *b*, unorganized agar; *c*, peritoneal reaction.

PLATE 6.

FIG. 12.—Reaction following ligature of pancreas and excision (Group B, 3 days). *W*, isolated pancreas acini; *X*, different stages in degenerative metaplasia; *Y*, change of acini to duct type; *Z*, normal pancreas.

FIG. 13.—Mitotic figure in acinus of pancreas (Group B, 10 days).

FIG. 14.—Pancreas cell inclusions (*i*) from Group B, 10 days.

FIG. 15.—Mitotic figure and cell inclusions (*A*) from Group A, 10 days.

PLATE 7.

FIG. 16.—General reaction Group A. *A*, normal pancreas; *B*, degenerative metaplasia; *C*, connective tissue.

FIG. 17.—Pancreas reaction Group A. *A*, duct type of acini; *B*, normal acini; *C*, connective tissue.

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